# A STUDY OF PARTIAL STERILITY IN CERTAIN HYBRIDS

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## INTRODUCTION

In 1909, I made a cross between *Nicotiana rustica* L. and *Nicotiana paniculata* L., in order to observe the behavior of the progeny of a hybrid in which the individuals of the first generation are only partially fertile.

These species were selected because the investigations of several early hybridizers had shown them to be particularly suitable for such a study.

The results obtained seem to be of considerable genetic interest, because of the indication from other sources that a high proportion,—perhaps a majority,—of the domestic plants and animals may have been derived from hybrids where the immediate cross is not completely fertile, in a somewhat similar manner. A short abstract of the work was published in 1915, but activities connected with the war have prevented a thorough consideration of the matter until now.

## THE TYPES USED

The strains of *N. rustica* L. under observation were received from Dr. O. COMES through the Office of Plant Introduction of the U. S. DEPARTMENT OF AGRICULTURE. They are evidently descendants of some of the

plants which he used in compiling his monograph on Nicotiana published in 1899.

*N. rustica* is well known as a robust annual, becoming biennial or triennial in sub-tropical regions, having cordate, sub-cordate, or ovate hairy leaves and a more or less branched panicle of greenish yellow flowers. Its origin is unknown, as it is said to have been cultivated over the greater part of North America in the time of COLUMBUS. It was the first species of Nicotiana to be taken to Europe, and the first to be cultivated by early Virginia colonists, though soon replaced by varieties of *N. tabacum* obtained from the West Indies and South America.

COMES (1899, p. 20) used practically the same description for the species as DECANDOLLE (Prodromus XIII, I. p. 563). This description, however, appears to be a generalization which will include all of the varieties grouped under the name, rather than a distinct type.

COMES accepts a division of the species into six varieties, of which he gives descriptions and figures; but it must be said that neither his words nor his drawings emphasize adequately the differences between them.

The differences are for the most part merely quantitative, it is true, but there are characters which, as extreme deviates, may be said to separate at least four types, *asiatica* to *jamaicensis*, *brasilia*, *texana* to *humilis*, and *scabra*. As a matter of record, I have produced a large number of pure lines from crosses between these types, which, when grown side by side, could easily be distinguished from each other in the field. But, as in most cases where one is dealing with a plant which has been under cultivation for a long period of time, the distinguishing marks bear description only in the case of the extremes.

Whether the varieties actually grown by COMES and sent out under the varietal names noted above, do in truth correspond with the types from which they were originally described, is uncertain. After examining all of the material in the GRAY HERBARIUM and the NATIONAL HERBARIUM, I am of the opinion they do not. But this has little bearing on a genetic investigation. The varieties received under the names *asiatica*, *brasilia*, *humilis*, *texana* and *scabra* were distinct. The variety termed *jamaicensis* was not markedly different from *asiatica*, when grown under optimum conditions; and when grown under poor conditions approached *humilis*.

These observations coincide with those of SETCHELL under Californian conditions, although COMES places *jamaicensis* nearer *texana*.

Those interested in the pure taxonomy of the subject should consult COMES's monograph of 1899, and SETCHELL's comments on plants raised from COMES's seed, published in 1912. I will simply describe the four types used in the experiments.

*N. rustica* var. *texana* (Naud., hort. Paris.) Comes.—Monographie Nicot., 21, t. IX. This variety seems to be the most generalized type of *N. rustica*. It is moderately branched, with comparatively long internodes, growing under different conditions from 1.25 m to 1.75 m high. The leaves are slightly rugose, ovate, and sometimes somewhat cordate. The handsome loose panicle is well filled with flowers. The copy of COMES's detail plate (figure 1) shows the characteristics of the flowers and fruit, although the corolla lobes are hardly as deeply cut or as sharply pointed as represented there. Figure 12, b, is representative.

*N. rustica* var. *brasilia* Schrank (Bot. Zeit. 1807, No. 17, p. 260).—Monographie Nicot., 22, t. XI. This variety compares with *texana* externally as does DE VRIES's mutant *gigas* with its progenitor *Oenothera*

FIGURE 1.—*Nicotiana rustica*, flowers and fruit. (1) *texana*, (2) *jamaicensis*, (3) *brasilia*, (4) *asiatica*, (5) *humilis*, (6) *scabra*. After COMES.

*Lamarckiana*. It is a heavy-set plant, a *rustica* with the parts telescoped together and enlarged. The stem is thick and angular from the large vascular bundles which go to the huge leaves. The plant is comparatively short, in typical strains hardly reaching 1 m, but the leaves are large, thick, and very rugose and bullate, showing more of a tendency to a cordate base than any other variety. As a commercial plant, it evidently outyields all other *rustica* varieties, for the broadly ovate leaves are often 50 cm long. The tendency to produce few lateral branches would also seem to be of some advantage in this respect. The stout panicle is so shortened that in the young plant it approaches a head. The flowers like the other parts are short and stout. COMES's detail drawings are corroborated by my own observations. Figure 10, b is characteristic of the young plant, but as the plant becomes older the inflorescence elongates somewhat.

*N. rustica* var. *humilis* Schrank (Bot. Zeit. 1807, No. 17, p. 260).—Monographie Nicot. 23, t. XIII. This variety may be said to be a small, robust *texana* of very early maturity. Though it seldom grows to more than 50 cm in height, with leaves in proportion, it is an exceedingly vigorous plant. The general habit of growth is very reminiscent of *texana*, but the leaves, flowers and fruit of a typical plant are easily distinguishable

FIGURE 2.—*N. rustica humilis* Schrank.

from those of other varieties. The leaves have a rather bright polished appearance, are somewhat fleshy, and tend toward an orbicular shape. The lobes of the corolla and the shape of the fruit also approach rotundity. Figure 2 is from a characteristic specimen.

*N. rustica* var. *scabra* (Cav.) Comes.—D. C. Prodr. XIII, I, p. 564.—Monographie Nicot. 23, t. XIV. Variety *scabra* may be considered as

somewhat the opposite of variety *brasilia*. It is tall, sometimes reaching 2.0 m, and though branching freely, is compact because of the decidedly ascendant tendency of the laterals. Leaves and stem are markedly pilose. As in SETCHELL'S specimens,

> "it lacks glands except on the flowering axes, being clothed elsewhere by a thick and compact covering of white slender hairs abruptly bent in the middle. Above, among the flowers these are mixed with the ordinary stalked, multicellular glands commonly found in the species Nicotiana."

The buds and young twigs have a dull purplish color which disappears as they develop. The panicles are loose, and rather delicate. Flowers keep on developing for several weeks even in the axes of mature fruits. The corolla tubes are narrower and the fruits more elliptical than in the other varieties. Figure 11, a, is typical.

The plants of *N. paniculata* L. used in these investigations were obtained from four different scources. One lot came from W. A. SETCHELL, (his number $\frac{10}{07}$): of its orgin I am ignorant. A second lot came from O. COMES. Other collections were made by C. T. BRUES and by W. E. CASTLE in Peru. The species does not seem to be variable, as each of these strains was so similar to the others that they might well be taken for samples of the same pure line.

*N. paniculata* L.—D. C. Prodr. XIII. I, p. 561. R. & P., Fl. Per., t. 129. This plant hardly needs description. It has been grown in all parts of the world since 1752 when it was discovered in Peru. Figure 3 is from a photograph of a typical plant. It is a low plant branching freely at the base, with cordate pubescent long-petioled leaves, and having long loose panicles of tubular flowers. The flowers are yellow-green, gibbous below the limb. The narrow limb is concave, and slightly fine-lobed. It may be reflexed when in full bloom.

## THE WORK OF THE EARLY HYBRIDIZERS

Though one cannot be certain as to the varieties of *N. rustica* used by the early hybridizers, there is no question but that the plants which formed the material for KÖLREUTER, SAGERET, NAUDIN, GODRON, GÄRTNER and FOCKE in their experiments with crosses between *N. rustica* and *N. paniculata*, did actually belong to the two species described above.

The cross *N. rustica* × *N. paniculata* has the honor of being the subject of the first investigation in plant genetics. It was made by KÖLREUTER in 1760, and the first hybrid generation studied the following year. The

reciprocal cross was not quite so easy to make, but, after some failures, it was also successfully grown in 1762.

The two species remained favorite subjects for study by the early plant hybridizers. HEDWIG, WIEGMANN, GÄRTNER, NAUDIN, GODRON and FOCKE, made more or less extended experiments with them, crossing and recrossing them in accordance with the methods in vogue before MENDEL's time. I have examined the papers of KÖLREUTER, WIEGMANN, GÄRTNER, NAUDIN and FOCKE, but will follow roughly the compilation made by FOCKE. The papers of HEDWIG and GODRON I have not been able to obtain.

The hybrids of *N. rustica* × *N. paniculata* were exactly the same as those of the reciprocal cross, although GÄRTNER found the one with *N. rustica* as the mother, to be somewhat more fruitful than the other.

KÖLREUTER and most of the other observers found a high degree of uniformity among the first generation progeny; but GÄRTNER obtained some examples which had shorter and broader flowers than usual, and was able to distinguish several hybrid types. FOCKE's hybrids, also, were variable, but the reason in his case (and possibly with GÄRTNER) was the use of several varieties of *N. rustica* in their production.

KÖLREUTER states that the hybrid plants were practically intermediate between the two parents in all characters, except that they were partially sterile. GÄRTNER, on the other hand, found them to have considerably more resemblance to *N. paniculata* than to *N. rustica*, especially in their rounded leaves, their stickiness and their long corollas. FOCKE's observations were just the opposite of GÄRTNER's. He had great difficulty in distinguishing the hybrids from *N. rustica*, but found little similarity to *N. paniculata* except in the habit of growth and the numerous glands. Measurement of the flowers of FOCKE's hybrids and their parents were as follows:

| | *N. rustica* | $F_1$ | *N. paniculata* |
|---|---|---|---|
| | mm | mm | mm |
| Length of corolla | 14 | 19 | 26 |
| Least diameter of corolla | 7 | 6 | 5 |
| Greatest diameter of corolla | 9 | 8 | 6 |
| Spread of corolla | 18 | 15 | 9 |

All of the hybridizers found the first-generation hybrids to be very vigorous and of early maturity. FOCKE obtained some dwarf specimens, however, although these blossomed 2 weeks before *N. rustica* and 4 weeks before *N. paniculata* sown at the same time.

FIGURE 3.—*N. paniculata* L.

KÖLREUTER concluded that the plants were more fertile with the pollen of either parent than with their own. He even found a higher degree of fertility with the pollen of a variety of *N. tabacum* known as *N. perennis* than when the plants were mated *inter se*. GÄRTNER's experiments showed the hybrids to be more easily back-crossed with pollen of *N. paniculata* than with that of *N. rustica*.

FOCKE states that he obtained seed by back-crossing with pollen from *N. Langsdorffii*, but the plants coming from these seeds reproduced the original hybrids. Perhaps he met here a phenomenon that I have observed when attempting to cross Nicotiana species which seldom produce hybrids. Seed is produced which gives again the maternal species. I have attributed the matter to polyembryony effected through the stimulus of the foreign pollen.

The hybridizers all seem to be in agreement that the plants of the second hybrid generation, produced by sib-matings of the partially sterile first hybrid generation, are extremely variable both in morphological characters and in fertility. KÖLREUTER, GÄRTNER, NAUDIN and FOCKE each found that the second-generation plants resembled *N. rustica* rather than *N. paniculata*, and they were also of the opinion that those plants which stood nearest to *N. rustica* were the most fertile. WIEGMANN, on the other hand, observed a similarity to *N. paniculata* in later generations.

NAUDIN obtained 12 second-generation individuals which differed very materially the one from the other, ranging from 30 cm to 120 cm in height. One plant had lanceolate leaves, something never observed in the parent species. These plants were stated to have been more fertile than those of the first hybrid generation. Plants of the third hybrid generation similar to *N. rustica* were found to be the most fertile.

## EXPERIMENTAL WORK

### *The parental strains and their behavior*

The first point of genetic importance to which attention should be called in connection with this material is the uniformity of the strains. Each of the four varieties of *N. rustica* and the strain of *N. paniculata* used in the crosses were selfed for two generations before any quantitative data were recorded. Presumably they had been self-fertilized either naturally or artificially for many generations before, if one may judge from the slight variability exhibited. *N. rustica texana* (348) was indeed somewhat variable in height, ranging from 36 to 75 inches (90 to 190 cm); but in habit of growth, shape and texture of leaves, size of flowers and fruit, it was very

uniform. *N. rustica scabra* (352), *N. rustica brasilia* (349), and *N. rustica humilis* (350) were likewise uniform in all characters, even in height. Table 1 shows height measurement on *N. rustica brasilia* and *N. rustica scabra*. The impression of variability given by the measurements is a little misleading, however, since a slight difference in height of the panicles was obliterated in the mind of the observer by the general similarity of the plants in their various characters.

The most uniform variety of *N. rustica* was the small strain known as *humilis*. In one season the range of height measurements on somewhat more than 100 plants was less than 15 cm, and visitors often remarked that they did not know it was possible to obtain a strain of plants so lacking in variability when grown in open field. Similar remarks were made about *N. paniculata* (331). Height measurements on a population of several

TABLE 1

*Height of crosses between Nicotiana rustica scabra (352) and N. rustica brasilia (349).*

| VARIETY OR CROSS | CLASS CENTERS IN INCHES | | | | | | | | | | | | | | | | | | | | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 | 63 | 66 | 69 | 72 | 75 | 78 | |
| 349 | | | | 4 | 10 | 22 | 14 | 7 | | | | | | | | | | | | | | | 57 |
| 352 | | | | | | | | | | 2 | 1 | 5 | 11 | 16 | 17 | 6 | | | | | | | 58 |
| 352 × 349 $F_1$ | | | | | | | | | | | | | 1 | 3 | 0 | 5 | 5 | 6 | 1 | 1 | | | 22 |
| 349 × 352 $F_1$ | | | | | | | | | | | | | | | 3 | 5 | 2 | 4 | 6 | 5 | 1 | 2 | 28 |
| (349 × 352)-1 $F_2$ | | | | 2 | 3 | 6 | 2 | 7 | 12 | 8 | 12 | 13 | 12 | 12 | 4 | 15 | 4 | 0 | 1 | 0 | 1 | | 114 |
| (349 × 352) 1-1 $F_3$ | 4 | 9 | 32 | 43 | 17 | 12 | 2 | | | | | | | | | | | | | | | | 119 |
| (349 × 352) 1-2 $F_3$ | | | | | | | | | 3 | 4 | 6 | 10 | 9 | 7 | 28 | 13 | 10 | 8 | 3 | | | | 101 |

score of plants usually exhibited a maximum range of less than 12 cm. The plants appeared as if they might have been cut out by a steel die.

Several crosses were made between the *N. rustica* varieties, and their behavior noted through three filial generations. These crosses were *N. rustica scabra* × *N. rustica brasilia*, *N. rustica texana* × *N. rustica brasilia*, *N. rustica scabra* × *N. rustica humilis* and *N. rustica texana* × *N. rustica humilis*. It seems unnecessary to describe this work at length. Three points are to be noted, however, as pertinent to the discussion of the species hybrids described later. (1) The reciprocal hybrids were very similar to each other. (2) The varieties differed from each other by many heritable factors. This is indicated by the greatly increased vigor of the $F_1$ generations, and by the extreme variability of the $F_2$ generations. (3) In no case could each of the four *rustica* varieties be reproduced from a single cross.

*N. rustica scabra* × *N. rustica brasilia.* In table 1, it is shown how much taller the $F_1$ plants were than the average between the two parents.

The $F_1$ generation average was even pushed considerably beyond the average of the taller parent (352). The different $F_1$ generations were very similar to each other in habit of growth, size of leaf, size of flower and size

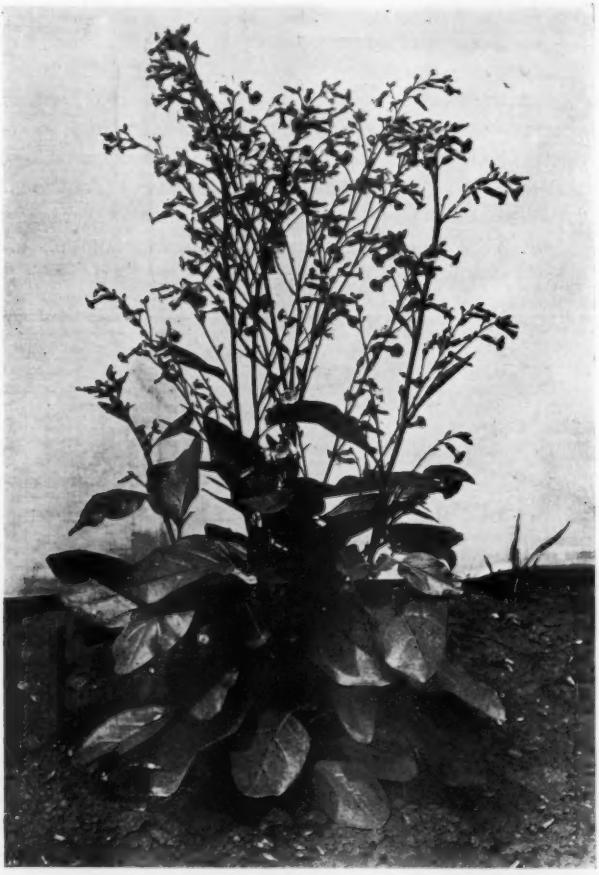

FIGURE 4.—*N. rustica humilis* × *N. paniculata*. $F_1$ plant.

of fruit. Height measurements indicated a slight difference in favor of the cross using *N. rustica brasilia* as the female parent, but the divergence is hardly enough to be significant even in that case.

The variability of one of the $F_2$ generations is illustrated very well by the height measurements. The possibility of obtaining strains rather uniform in height by selecting extreme $F_2$ individuals is also manifest.

The most important fact connected with this cross, however, and with the other crosses as well, cannot be demonstrated statistically without covering too much space. It is this: The $F_2$ individuals, though variable, were what might have been expected by a plant breeder familiar with the two strains used in the cross. They exhibited recombination of the existing characters. Plants similar to *N. rustica texana* were found, but no plants similar to *N. rustica humilis*. Presumably *texana*-like plants arose from the following combinations: Loss of *scabra* hairs; non-appearance of *brasilia* habit of growth, with nevertheless a distinct modification of the *scabra* habit; absence of bullate leaves; and an intermediate size and shape of flowers and fruit, verging toward those of *scabra*. The peculiar smooth fleshy leaves of *N. rustica humilis* did not appear in any members of the $F_2$ generation.

It might also be mentioned here, that the *gigas*-like habit of *N. rustica brasilia* appeared to be a simple recessive, but it was so changed by numerous modifiers as to make classification difficult.

*N. rustica texana* × *N. rustica brasilia*. In this cross the same vigorous $F_1$ generation and variable $F_2$ generation appeared. No *humilis*-like or *scabra*-like plants were found. Neither the fleshy leaf of the former nor the characteristic hairs of the latter appeared, though certain individuals had small narrow-tubed flowers and oval capsules much resembling *scabra*.

*N. rustica scabra* × *N. rustica humilis*. The height of the $F_1$ individuals in this cross, unlike that in the first two crosses, was somewhat less than that of the taller parent. The $F_2$ was again extremely variable. *Humilis*-like plants were regained, and *texana*-like plants appeared; but no plants resembling *brasilia* were found. The appearance of plants similar to *N. rustica texana* was not unexpected, for given the absence of the *scabra* hairs, the characteristics of *texana* are somewhat intermediate between *scabra* and *humilis*.

*N. rustica texana* × *N. rustica humilis*. This cross was similar to the preceding one except that the characteristic hairs of *scabra* were absent as well as the *gigas*-like characters of *brasilia*.

The general conclusion from this work is that the four *N. rustica* varieties studied differ the one from the other in a comparatively large number of heritable characters; but that by crossing any two of them, the characteristics of both the others *can not be reproduced*.

### *Nicotiana rustica humilis crossed with Nicotiana paniculata*
### *Experiment 1*

*Nicotiana rustica humilis* (350) crossed with *N. paniculata* (331) was the hybrid selected for particular study.

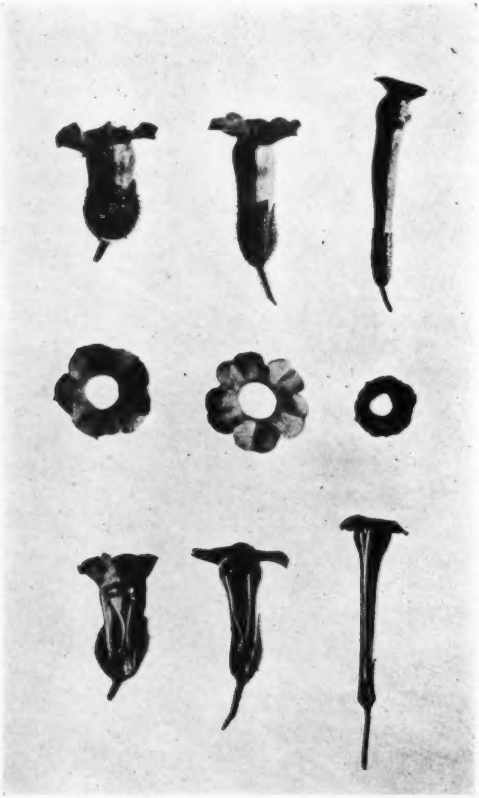

FIGURE 5.—At left, *N. rustica humilis;* center, *N. rustica humilis* × *N. paniculata* ($F_1$); at right, *N. paniculata.*

Hybrids between *N. paniculata* and two other varieties of *N. rustica*, viz., *brasilia* and *scabra*, were made successfully, but were not carried beyond the second hybrid generation. The only point to be emphasized now in regard to them is that they were extremely uniform in all characters.

The reciprocal cross with *N. rustica humilis* was also made. This hybrid was not quite so easy to obtain as when the parentage was reversed, but the only difference noticeable in the reciprocal $F_1$ populations was a slightly taller plant in the case of *N. rustica humilis* ♀ × *N. paniculata* ♂.

My first $F_1$ population of the cross *N. rustica humilis* (350) × *N. paniculata* (331) was grown in 1910. In leaf size, shape and texture, and in shape of corolla, the plants were very similar to *N. rustica humilis*. In fact, any taxonomist would have classified them as *N. rustica* plants.

But when examined carefully it was seen that they showed the influence of the *N. paniculata* parent, particularly in the intermediate length of corolla and in the spreading habit of growth,—the branches ending in loose *paniculata*-like panicles. These characteristics are probably better

TABLE 2

*Variations in length of corolla tube in cross between N. rustica humilis (350) and N. paniculata (331).*

| MATERIAL | CLASS CENTERS IN CENTIMETERS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1.125 | 1.375 | 1.625 | 1.875 | 2.125 | 2.375 | 2.625 | 2.875 | 3.125 | 3.375 |
| 350, plant 1 (1910) | | 24 | 1 | | | | | | | |
| 350, plant 2 (1910) | 1 | 23 | 1 | | | | | | | |
| 350, 5 flowers each (1910) | 33 | 168 | 63 | | | | | | | |
| 331, plant 1 (1910) | | | | | | 2 | 22 | 3 | | |
| 331, plant 1 (1910) | | | | | | | 24 | 1 | | |
| 331, 5 flowers each (1910) | | | | | | 47 | 178 | 40 | | |
| (350 × 331) $F_1$, plant 1 (1910) | | | | 24 | 1 | | | | | |
| (350 × 331) $F_1$, 5 flowers each (1910) | | 2 | 9 | 208 | 63 | | | | | |
| (350 × 331) $F_2$, 1 flower each (1911) | 2 | 7 | 31 | 90 | 76 | 24 | 7 | 3 | 1 | 1 |

shown in figures 4 and 5 than they would be by any amount of description and measurement.

I have already spoken of the uniformity of the two parental types in all characters. The $F_1$ plants were just as uniform in their traits, though they were about 25 percent taller than *Nicotiana paniculata* (the taller parent). Table 2 gives an illustration of this remarkable lack of variability.

The $F_1$ plants produced only from 1 to 30 seeds per capsule when crossed *inter se* or when self-pollinated, even though considerable quantities of pollen were applied. This sterility seemed to be equally a sterility of ovules and of pollen for no greater quantities of seed were produced when pollen of the $F_1$ plants was applied to either parent, or when the pollen of either parent was applied to them.

Matings 331 × (350 × 331) $F_1$ and 350 × (350 × 331) $F_1$ were somewhat difficult to obtain, but after a score or so of attempts to use $F_1$ pollen on each of the two pure species, several capsules averaging 10 seeds each resulted. The reciprocal crosses, i.e., the use of the pollen of the pure strains upon the flowers of the $F_1$ plants, were usually successful, the capsules ranging from 3 to 22 seeds each. Perhaps the very fact that back-crosses with the $F_1$ pollen were harder to obtain than the reciprocals, shows that ovule sterility was less than pollen sterility. But when they matured at all, the back-crossed capsules of each of the four combinations possible contained about the same quantity of seed. Consequently it seems reasonable to attribute this apparent functional difference between the ovules and the pollen of $F_1$ plants, to the greater protection against inclement conditions received by the ovules, rather than to inherently greater fertility.

The plants of both (350 × 331) × 331 and (350 × 331) × 350 showed the general uniformity of the true $F_1$ generation, though the former were considerably more variable. The back-cross with *N. paniculata* (331) took on somewhat more of the *paniculata* characters, and that with *N. rustica* (350) resembled *rustica* more than did the $F_1$ generation. For example, the flowers of the *paniculata* back-cross averaged about 2.3 cm by 65 cm in length and breadth of the corolla tube, while those of the *rustica* back-cross averaged 1.9 cm by 0.9 cm.

By exercising great care in pollinating two $F_1$ plants *inter se*, a quantity of seed was obtained from which 246 plants were raised in 1911. The percent of germination of the seed is not known.

The $F_2$ plants were each unlike all the others though in general they resembled *N. rustica* more than *N. paniculata*. In height they varied from 24 cm to 170 cm. Flowers from 21 plants are shown in figure 6 compared with flowers of the two grandparents in the upper right-hand and lower left-hand corners respectively. It is clear that all sorts of combinations between the long narrow corolla of *N. paniculata* and the short broad corolla of *N. rustica* are in evidence, but the point of particular interest is that the extremes exceeded those of each parent. The same point is emphasized by figure 7, in which the largest leaves of a number of $F_2$ plants are shown on the same scale.

A good many measurements of the various characters of this $F_2$ generation were made, but reports of series of measurements on particular characters serve to confuse rather than to clarify the points which seem important. For this reason a number of photographs of $F_2$ plants are submitted in place of detailed measurements.

Though nearly all of the plants of this generation would have been grouped as *rustica* plants, there were seven which resembled *paniculata* rather closely. Of these, four retained traces of *rustica* descent. The other three plants duplicated *N. paniculata* exactly. Figure 8 shows one of these segregates. By comparing it with figure 3, a representative of the pure species, one can see that it is no exaggeration to use the word exactly.

In every feature of habit of growth, of leaf and of flower, these plants were wholly *N. paniculata*.

Naturally the suspicion arose that these plants were *paniculata* individuals which had been included by mistake, or had arisen as volunteers. Nevertheless there was some evidence this was not the case. In the first

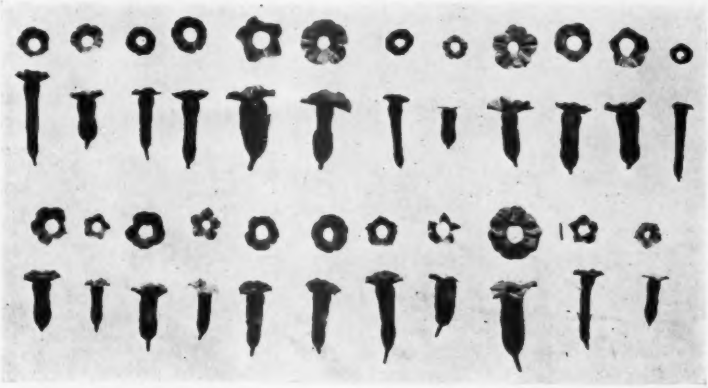

FIGURE 6.—Flowers from 21 plants of $F_2$ generation *N. rustica humilis* × *N. paniculata*. Upper right, *N. paniculata;* lower left, *N. rustica humilis*.

place, the seeds from which the plants were grown, were planted in sterilized soil, and the seedlings transplanted very carefully and tagged in the field. Some misplacement was found, of course, for no one can carry on extended experiments without error. But on examining the field of some 10,000 plants, the traceable misplacement was found to be about 1 plant per 2000, while here were three plants in a lot of 246 for which to account. In the second place, though two of these *paniculata*-like plants apparently were as fertile as the pure-line *paniculatas, one was completely sterile.* The fact that two of these supposed segregates were so fertile is admittedly a somewhat suspicious circumstance, but the appearance of a sterile plant duplicating *paniculata* characters seemed to clinch the claim of that particular individual as a segregate from the original cross.

Seed from self-fertilized flowers of one of the fertile plants was grown the next year, and the progeny bred true to the *paniculata* characters.

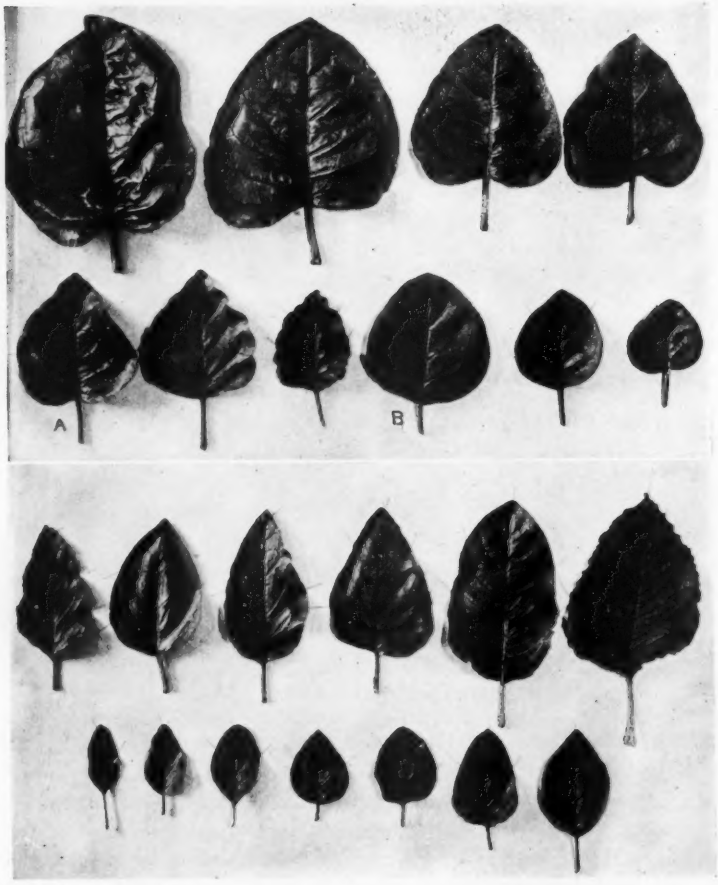


FIGURE 7.—The largest leaf on each of the 21 plants of $F_2$ generation *N. rustica humilis* × *N. paniculata* of which the flowers are shown in figure 6. A, *N. paniculata;* B, *N. rustica humilis.*

Not a trace of influence of *N. rustica* appeared. This fact combined with the others mentioned made me so apprehensive of the whole matter that the experiment was repeated in its entirety. This second investi-

gation of the *rustica-paniculata* cross is described later in the paper. Suffice it to say now, that I am convinced from it that the three *paniculata*-like plants of the $F_2$ generation under discussion were in fact segregates.

Several reasons for accepting this view will appear later. We will mention here only the reason why the complete fertility of the supposed segregate need not be taken as a particularly suspicious circumstance. It is this: Though the $F_1$ plants were very slightly fertile, their fertility was uniform. The $F_2$ plants, on the other hand, showed as great a variability in the fertility exhibited as in any other character complex. Several plants could not be induced to set a single seed in the limited number of trials made, either with their own pollen, with pollen of any of their sibs, or with pollen from either of the species entering into the cross. In others, there were varying degrees of fertility up to complete fertility as far as the quotas of seed set are concerned, though some small percentage of pollen abortion was found in the best.

Among the plants of the $F_2$ generation, 6 or 7 were found which reproduced the characters of *N. rustica humilis*, one of which is illustrated in figure 9. None of them was markedly sterile, though varying degrees of fertility were exhibited. But the fact that *N. rustica humilis* was reproduced as a segregate need not astonish one. The astonishing fact was that, though variety *humilis* entered into the cross, and though variety *humilis* crossed with other varieties of *N. rustica* did not throw segregates duplicating varieties *brasilia* and *scabra*, nevertheless *these varieties were duplicated* in the segregates when *humilis* was crossed with *paniculata*.

Figure 10 shows an $F_2$ segregate of this population at the left, compared with a *brasilia* plant at the right. Though the photographs do not emphasize the points of similarity, in the field they were very marked. This plant could only have been classified as a *brasilia* plant, though the leaves were not so bullate as in the particular pure-line *brasilia* plant with which we have chosen to compare it. On the other hand, several other pure lines of *brasilia* plants have been grown which do not exhibit such a bullate appearance as the one here shown; and further, the particular way the sunlight fell on this plant while the photograph was being taken, rather obscures the amount of leaf-wrinkling it possessed.

In figure 11 a typical plant of variety *scabra* is shown at the left, while a *scabra*-like segregate appears at the right. In this picture, one who is familiar with the appearance of *scabra* will be struck with the fact that the segregate seems to be more strikingly *scabra*-like than the plant from the pure line. This is because of the fact that the photograph of the true *scabra* plant is so sharply focussed that the grayish appearance due to the

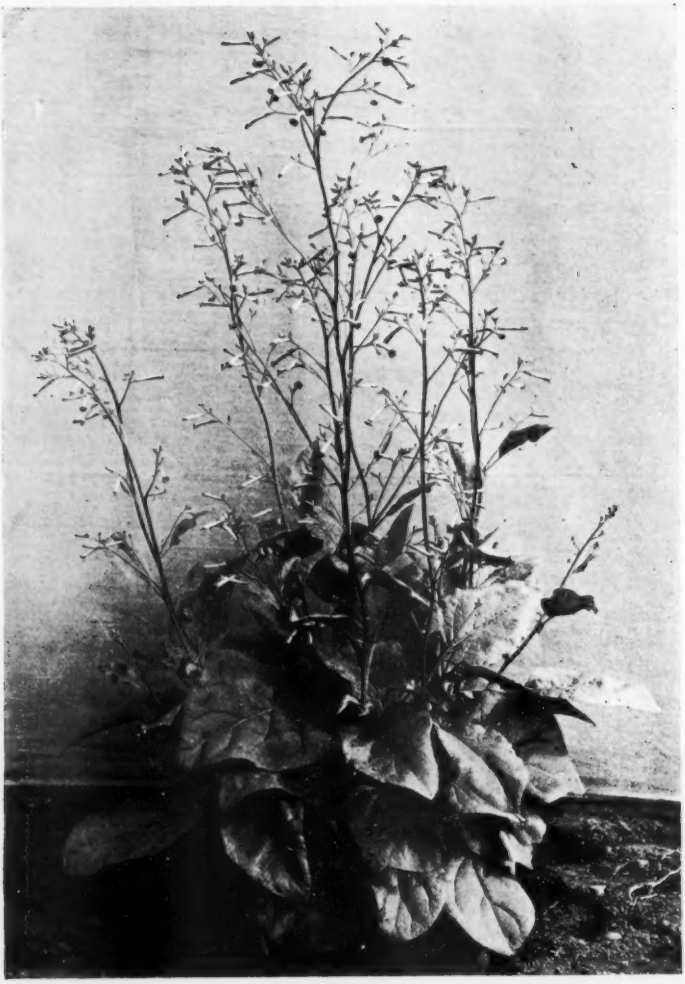

FIGURE 8.—$F_2$ segregate, *N. rustica humilis* × *N. paniculata*, duplicating characters of *N. paniculata*.

hairs is partly obliterated. The habits of growth may seem somewhat different but this is not really true. The segregate is a little more vigorous than the *scabra* plant, and though the photographs were taken when the plants were of the same age, the lateral branches of the *scabra* plant had not developed to the same extent. In another week the pictures would have been practically identical, for the two plants tallied character by character even to the characteristic dull purple in the young flower buds.

Again, we have a third segregate in the left-hand plant of figure 12 which might have been die-cut as a representative of variety *texana* (the right-hand plant). The similarity of the two plants is obvious even in the photographs taken under different weather conditions. Notice specially the size and shape of the flowers, the method of branching, and the character of the panicles. The leaves are the same shape, size and texture, and are set at the same angle on the stalks.

A few other extreme plants are interesting in this connection. A rather gigantic form is pictured in figure 13. It is not extremely tall, as can be seen by comparing it with the foot-rule, but the leaves are far larger than any we have examined in the larger cultivated types of *N. rustica*. The largest leaf measured possessed a blade 75 cm × 60 cm. Contrast this huge plant with the little one shown in figure 14, where the whole plant is only 25 cm high and has a maximum leaf size of 9 cm. Oddly enough the size of the flowers is not noticeably correlated with the size of the plant as a whole. The flowers of the lilliputian of figure 14 are almost as large as those of the giant sister of figure 13. Another peculiar type is illustrated in figure 15. In texture and shape of leaf, this plant is much like the *brasilia* variety; but the inflorescence is unique. The lateral branches combine with the main stem to produce the appearance of one large corymb. In marked contrast to this type is one shown in figure 16 where the prominently white-veined leaves curl at the edges and the inflorescence is such that the plant has a pyramidal appearance. Again in figure 17, a plant is shown which is quite different from any described variety of *N. rustica*. The sparse leaves are lanceolate in shape and very delicate in texture; the flowers are long and narrow; and the general character of the panicle is much looser than in the other types described, although approached by *N. rustica scabra*.

The photographs of the few $F_2$ individuals shown in the figures, represent some of the extreme types. Now in imagination, picture graded series of intermediates between these particular plants—hardly two of similar appearance—and you have a fair idea of the $F_2$ generation of this cross.

It is useless to try to show frequency distributions of particular characters. They fail to serve the purpose, for it is the *combination* of *characters* which makes every plant distinct from the other. At the same time the measurements of height of plant, length and breadth of leaves, size of flower and of fruit, show clearly four facts which are important for

FIGURE 9.—$F_2$ segregate, *N. rustica humilis* × *N. paniculata*, duplicating characters of *N. rustica humilis*.

the theoretical discussion which is to be given later. (1) In an $F_2$ series of less than 300 plants, nearly every individual *seems* to have an hereditary constitution different from all others. (2) In height of plant and in size and shape of leaves, the range is far beyond that of either grandparent; but in size and shape of flower, though combinations of the grandparental

characters are found, they do not pass far beyond those actually existent in the pure species. (3) Nearly all of the plants resemble *N. rustica*. Those which resemble *N. paniculata* at all, are almost identical with it. In other words, a series of combinations presumably possible, appears to have been omitted. (4) The fertility of the plants varies from a condition where almost all of the pollen is abortive and few if any seeds are produced (limited number of trials) with sib pollen or with pollen of either of the grandparental species, to a condition where every ovule (full capsules) seems to function with "own" pollen (though from 20 percent to 30 percent of this pollen is abortive).

### *Nicotiana rustica humilis crossed with Nicotiana paniculata*
### *Experiment* 2

Owing partly to the suspicion that the *paniculata*-like segregates in the $F_2$ population of the cross just described might have been "volunteers," and partly to the desire to make some observations that had been omitted, a repetition of the cross between *N. rustica humilis* (350) and *N. paniculata* (331) was made.

The $F_1$ generation was practically identical with that described under experiment 1. The plants were slightly taller and the flowers slightly larger, but this difference may be attributed to the rich soil upon which they were grown.

Some considerable time was spent studying the fertility of this generation, with the following results. From 1 percent to 7 percent of the ovules were proved to be functional, by careful pollinations with "own" pollen, with sib pollen and with pollen of the two parental species. This ratio was determined by comparing the number of seeds actually produced with the number found in full capsules of *N. paniculata*, as a determination of the number of ovules in a flower of the plants under consideration was difficult. There is some inaccuracy in these figures, therefore, but it is probably not very great. The variation found is due, in part, to manipulation difficulties and in part to the small samples involved; and one may take it that under the best conditions 3 or 4 percent of the ovules of the $F_1$ generation on an average will produce seeds, and that these will germinate in from 35 to 55 percent of the cases.

Samples of the pollen of $F_1$, taken from mature anthers, likewise showed a variable ratio of grains apparently perfect. The variation here was from less than 3 percent to about 10 percent, and seems to be due largely to the great difficulty of obtaining random samples. When pollen from anthers

FIGURE 10.—At right, *N. rustica brasilia;* at left, $F_2$ segregate, *N. rustica humilis* × *N. paniculata,* duplicating characters of *N. rustica brasilia* with the exception of the bullate leaves.

just opened is shaken out, the count is distorted because the perfect grains shake out more easily. When the pollen is scraped out with a scalpel the results are a little more uniform, but even with this method they are not to be relied upon. Paraffine sections of mature unopened anthers gave a lower ratio—from 1 to 3 percent—of grains perfect in appearance; but this method also has its drawbacks. Sections from anthers of different phases of maturity, indicate that *many of the pollen grains which appear to be functional in the young unopened anther, dry up as soon as the latter opens*. Furthermore, germination tests made in stigmatic fluid and in sugar solutions, showed that from 20 to 70 percent of the grains which appeared to be perfect would not extrude pollen tubes. It may be argued that such a test does not preclude the possibility of a high percentage of germination of the "good" grains on the plant stigmas. This is true. But experience with the pollen of *N. rustica humilis* and *N. paniculata* in hanging-drop cultures, leads me to believe that over 80 percent germination can be obtained by this means, if the pollen is really functional.

The general conclusion from observations on the pollen of the $F_1$ generation of this cross, is that less than one-tenth of one percent of the tetrads which the pollen mother cells *start* to form, ever succeed in functioning as pollen.

One other set of observations should be mentioned in this connection, though the conclusions may be revised after more extended investigation. Examination of slides of *N. rustica humilis*, of *N. paniculata*, and of the $F_1$, indicates that each species has 24 chromosomes as the gametic number, and that a high percentage of breakdown occurs at the heterotypic division of the pollen mother cells.

Of the $F_2$ generation, 300 plants were raised to maturity. These plants were studied rather carefully, and it is hoped that some day the data may be punched on cards and all of the statistical relations made out with a Hollerith machine. For the present purpose too voluminous citation of these details regarding separate characters would simply result in obscuring the important points.

The plants were again extremely variable. For example, the height frequencies in decimeter classes, for the 288 plants which had reached their maximum height at the time of measurement, were as follows:

| Class centers in dm | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Frequency | 1 | 2 | 1 | 2 | 7 | 10 | 11 | 13 | 19 | 35 | 39 | 35 | 31 | 29 | 17 | 15 | 13 | 2 | 3 | 2 | 1 |

When one realizes that nearly all of the $F_1$ plants are included in *one* of these decimeter classes,—less than 10 percent falling into contiguous classes,—this range of the $F_2$ generation from 3 decimeters to 23 decimeters is tremendous. A similar variation occurred in habit of growth and time of maturity (range of 30 days), in shape, color, leafiness and hairiness of stem, in size, shape, thickness, bullation and hairiness of leaves, and in character of inflorescence (from the head-like panicle characteristic of variety *brasilia* to a loose panicle resembling a raceme). The fruit also showed extreme variation, ranging from a pumpkin shape, one and one-half times as broad as long, to an ovoid type nearly twice as long as broad, and from a length of 3 mm to a length of 18 mm. It is probable, however, that variation in fertility distorted these records. In flower shape and size, variation was not so marked. A few plants had *rustica*-shaped flowers longer than those characteristic of *paniculata* and a few had *rustica*-shaped flowers about one-half the size of those of *N. rustica humilis*; but only one plant approached *N. paniculata* flower characters.

The characters of the plants considered together showed that many of the plants could be classified as resembling varieties *humilis*, *brasilia*, *scabra*, and *texana* as in experiment 1. There were others which resembled those figured previously, as well as a few new types. Only one plant resembled *paniculata* at all closely, though certain features possessed by many individuals, length of corolla, hairiness, cordate leaves, etc., may have been hereditary contributions from this source. The one *paniculata* segregate, curiously enough, was only about two-thirds the size of a normal *paniculata*, though all of the size relations appeared to be the same,—that is, leaves and flowers were smaller in their proper ratios. The plant was 66 cm in height; the leaves were the exact shape, texture and hairiness of *paniculata*; the flowers also were exactly what one might imagine to be characteristic of a dwarf *paniculata*,—19 mm long and 3 mm broad, flaring at the end to 6 mm. About 20 percent of the pollen grains appeared to be normal, but the many attempts at self-pollination were all failures.

The appearance of this plant in this population, is to some extent a confirmation of the results of experiment 1. It gives more confidence in the supposition that the three plants apparently identical with *N. paniculata* found in that $F_2$ population, were in truth segregates from the $F_1$ generation.

From the standpoint of genetic theory, the questions connected with the fertility of this family seemed important, and a serious effort was made to discover the facts. Since the inquiries were subject to the limitations already described, the effort was not all that might be desired; nevertheless,

I believe that the data have a decided value, and that the conclusions drawn from them are satisfactory from the standpoint of statistical theory.

First, the pollen from 52 plants was examined microscopically. Representatives from all of the more distinctive types were included. The pollen was examined dry, in water and in glycerin. The general results are as follow:

(1) Judging from the amount of pollen formed and from the percentage of aborted grains, it appears uncertain that any $F_2$ plants are more sterile

FIGURE 11.—At left, *N. rustica scabra;* at right, $F_2$ segregate, *N. rustica humilis* × *N. paniculata*, duplicating characters of *N. rustica scabra*.

than the plants of the $F_1$ generation. In nine plants out of 52 examined, the proportion of pollen formed and the proportion of grains apparently viable were comparable with the proportions found in $F_1$. The grains apparently viable were between 3 percent and 10 percent of the total.

In one other plant, only aborted pollen grains were found, but it is assumed that this condition was not due wholly to heredity because the plant formed about 25 percent of the number of seeds expected normally when pollinated with sib pollen.

(2) Ninety percent of the plants produced much greater complements of good pollen than those of the $F_1$ generation, and the percentage of grains apparently viable ranged from 10 percent to 80 percent. No plants in this generation produced a proportion of "good" pollen equal to that of the pure species (95 percent to 98 percent), but three plants of the $F_2$ generation described in experiment 1 had over 96 percent "good" pollen. The modal condition was around 65 percent "good" pollen.

FIGURE 12.—At right, *N. rustica texana;* at left, $F_2$ segregate, *N. rustica humilis* × *N. paniculata*, duplicating characters of *N. rustica texana*.

(3) No decided correlation between plant characters and fertility as determined by this method could be discovered.

(4) No pollen germination tests were made on this population, either in stigmatic fluid or in sugar solutions, but judging from the complements of seed formed naturally (probably sib-pollination), many of the "good" pollen grains did not function. This conclusion was drawn from the fact that no seed was obtained on several plants producing some apparently

"good" pollen. It might be concluded that this result came about because of a greater proportion of sterility among the female gametes, but the general evidence is against such a conclusion.

The shortcomings of the pollen-examination method of estimating fertility are obvious. Because of them another method was used. It has its faults, but I believe that by its use a reasonably accurate comparison of the fertility of the $F_1$ and $F_2$ generations can be made, and this is the essential point.

Each plant was examined carefully at the stage when the seeds are just beginning to turn brown. The flowers had had a fairly equal chance of being pollinated naturally with sib pollen; and the seeds formed had matured sufficiently to give a fair idea of those retaining some measure of vitality, since abortive seeds had begun to shrivel. The procedure was as follows. The percentage of capsules formed per plant having been given weight in the judgment, several capsules from each individual were carefully "peeled" and an estimate of the fertility made from the seeds therein. The plants were thrown into eleven fertility classes ranging

TABLE 3

*Estimated fertility of the $F_2$ generation (350 × 331) through an examination of the capsules.*

| Class | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Frequency | 4 | 4 | 11 | 23 | 34 | 20 | 41 | 32 | 56 | 28 | 17 |

from 0 to 10. It may be assumed that classes 5 to 6 represent about a half complement of seeds, class 10 represents complete fertility on the female side, and class 0 represents complete sterility. The results of an examination of 269 plants are given in table 3.

The curve is not as smooth as it would have been, had not the records been taken in classes numbered 1, $1\frac{1}{2}$, 2, $2\frac{1}{2}$, etc. The tendency was to enter the records in the integral classes. But this fact does not interfere with the main result. On this scale the plants of $F_1$ belong uniformly in class 2. In other words, the $F_2$ plants were variable in fertility, but 97 percent of them showed greater fertility than those of the $F_1$ generation. At least 12 percent of the plants gave full complements of seed which seemed perfect in every way.

On the other hand, one *can not* conclude that a single plant—through heredity—was less fertile than the $F_1$ generation, *though such may be the case.* Let us look at the matter a little more carefully. Suppose it be admitted that the $F_1$ plants showed a slightly greater fertility than class 2. This would leave 19 plants out of 269 $F_2$ plants apparently less fertile

than those of the $F_1$ generation. At the same time it must be remembered that the $F_1$ plants were pollinated very carefully. A great deal of pollen was applied, and hundreds of flowers were tested. A similarly extensive test on each of these $F_2$ plants was impossible. Each plant self-pollinated (162) had only 5 flowers manipulated (with the exception of the *paniculata*-like plant), taking the usual precautions, though the capsules pollinated naturally were examined carefully. To the best of our knowledge and belief, therefore, evidence for a fertility less than that of $F_1$ exists for only the 8 plants of the first two classes, *and this evidence is not conclusive*, except possibly in the above-mentioned *paniculata*-like individual. In the first place, each of these plants might have produced *some* seed if pollinated as carefully and extensively as were our $F_1$ plants, since each produced as great a percentage of apparently viable pollen as the average $F_1$ plant. In the second place, in any family of plants individuals appear occasionally that are completely sterile for reasons which seem to be unconnected with heredity. Some of these plants may be of this type. It seems to me, therefore, that the following conclusion is in accordance with all the facts. *Ninety-seven percent of the $F_2$ plants produced in the cross between N. rustica humilis and N. paniculata were more fertile than the $F_1$ plants, and it is not certain that the remaining 3 percent were less fertile.*

One hundred sixty-two of these $F_2$ plants were self-pollinated. In almost every case 5 flowers were used. Unfortunately the prevalence of aphis and an excess of rain and wind during the remainder of the season ruined a very large part of this work. Only 94 plants matured seed, and of this number only 70 produced 75 or more seeds. Seed was collected from 203 plants where natural pollination had taken place, however, and the germination of each lot tested. One hundred seeds from each plant were counted, placed in separate Petri dishes on moist blotting paper, and kept in a rather warm greenhouse. The results were rather interesting. While most of the seeds—particularly the seeds of those plants which showed a high percentage of germination—sprouted after 4 or 5 days, a number of the dishes showed a continuous germination week by week,—several seeds germinating after 63 days. It would have been decidedly worth while to have traced the life history of some of these plants germinating at different periods to see if there were marked differences in their characters, but this was impossible.

Table 4 is a correlation table made up from the germination tests of 67 plants from which both selfed seed and open-pollinated seed were obtained. To those who know how uniform germination tests made from duplicate samples of seed usually are, it may seem strange that the corre-

lation between the two sets was only $0.33 \pm 0.07$. But this is not really so odd. The conditions under which the selfed seed matured were bad, but not uniformly bad. Some bags had aphides under them, others were free from aphides; some were wet on the inside, others stood up well and were only wet on the outside. Nevertheless, the results here and in table 6, show that many of these plants were in a rather balanced condition as regards maturing seed. Little changes in environment evidently affected

TABLE 4

*Correlation in percent of germination between open-pollinated and self-pollinated seed produced by $F_2$ plants of cross between N. rustica humilis and N. paniculata.*

| Percent self-pollinated | Percent open-pollinated: 5 | 15 | 25 | 35 | 45 | 55 | 65 | 75 | 85 | |
|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 1 | 3 | 1 | | 2 | 1 | 1 | 1 | | 10 |
| 15 | 2 | 2 | 2 | 3 | 1 | 2 | 2 | | | 14 |
| 25 | | 1 | | 3 | 4 | | | | | 8 |
| 35 | 1 | 1 | 1 | | 3 | | | | | 6 |
| 45 | | 2 | | 1 | 2 | | 2 | | | 7 |
| 55 | | 1 | 1 | | 1 | 2 | 1 | | | 6 |
| 65 | 1 | 1 | 1 | 1 | | | 2 | | | 6 |
| 75 | | 1 | | | 2 | | 3 | 1 | 3 | 10 |
| | 5 | 12 | 6 | 8 | 15 | 5 | 11 | 2 | 3 | 67 |

$r = 0.33 \pm 0.07$

TABLE 5

*Percent of germination of open-pollinated seed produced by plants of the $F_2$ generation of cross between N. rustica humilis and N. paniculata.*

| Percent germination | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 60 | 65 | 70 | 75 | 80 | 85 | 90 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Frequency | 16 | 17 | 14 | 17 | 19 | 12 | 13 | 15 | 17 | 9 | 13 | 10 | 9 | 11 | 5 | 1 | 3 | 2 |

the maturing capsules much more than similar changes would have affected pure lines, since several pure lines under observation at the same time gave germination tests of over 90 percent. Thus there is pretty good evidence for supposing that zygotes were eliminated with a high degree of frequency by external conditions; and it seems to me that it is more than a guess to say that there was a selective elimination of plants with particular hereditary complexes.

Table 5 gives the percent of germination of the open-pollinated seed.

*Cross between Nicotiana rustica scabra and N. paniculata. Experiment 3*

Before continuing to describe the descendants of the $F_2$ generation of the cross between *N. rustica humilis* and *N. paniculata* (experiment 2) two crosses between *N. paniculata* and other varieties of *N. rustica* should be discussed for the sake of comparison. The first of these is one in which *N. rustica scabra* was used as the female.

Both when the plants are considered as units and when specific characters are examined, the $F_1$ population compares in uniformity favorably with those already described. For example the height frequencies of 49 plants in decimeter classes starting with 7.5 are 6, 15, 19 and 9. Again the flower-length frequencies in millimeter classes beginning with 19 are 4, 20, 22 and 3.

The fecundity of the plants seemed to be slightly greater than in the other crosses, in that a larger percentage of self-pollinated flowers produced some seeds. But since the number of seeds per capsule obtained was about the same, it is not certain that the gross fertility exhibited was any greater.

The 257 $F_2$ plants raised to maturity showed again an extreme variability, ranging from 30 cm to 210 cm in height, and reproducing *brasilia*, *scabra*, *texana*, *asiatica*, *humilis*, *nana* and *gigas* types as before.

Three differences between the plants of this cross and those of the cross into which variety *humilis* entered were found, however, and these differences appear to be important. (1) *All* of the plants leaned decidedly toward the *rustica* side of the house. Not a single plant was found that one could say truthfully was *paniculata*-like, although traces of possible *paniculata* influence could be seen in fully half of the population. (2) Small plants with a starved appearance were found in considerable numbers, (38 out of 257 plants). In the cross between *N. rustica humilis* and *N. paniculata* scarcely two plants were of the same type, yet here 15 percent of the population were dwarfs that were fairly uniform in appearance. These plants resembled the one reproduced in figure 14, except that few seed capsules were formed. Flowers pollinated carefully by hand, as well as flowers pollinated by insects, fell off after 3 or 4 days. Toward the end of the season a few capsules were formed, but they contained no normal seed. A few collapsed seeds in which no embryo could be discovered, were all I obtained for my trouble. Pollen from these plants examined in glycerin appeared to have as high as 3 percent of normal grains, but there was no evidence that this appearance was a true criterion of vitality. *A part of these plants was tested carefully, and I believe it safe to say that they were less fertile than those of the $F_1$ generation.* (3) Among the other plants of the population a varying degree of fertility was found, with the

tendency toward high fertility as in experiment 2; but none showed decided evidence of being less fertile than the plants of the $F_1$ generation. Thus there was a high correlation between a physical type (the sickly dwarf), and sterility. Perhaps the phenomenon met here is due to a lethal factor simi-

FIGURE 13.—Giant $F_2$ segregate *N. rustica humilis* × *N. paniculata.*

lar to those described by MORGAN for *Drosophila melanogoster;* at least one may suppose it is not the same type of absolute sterility found to be questionable in the $F_2$ generation of experiment 2. This experiment was carried no further.

*Cross between Nicotiana rustica brasilia and N. paniculata. Experiment 4*

The cross in which *N. rustica brasilia* entered as the maternal parent, was very similar to that in which variety *humilis* was used as the *rustica* parent. Time for studying this cross in detail was not available, but 26 plants of the $F_1$ generation and 57 plants of the $F_2$ generation were grown and examined. The uniformity in physical appearance and in fertility characteristic of the other $F_1$ generations was again found here. Similarly also the $F_2$ generation was extraordinarily variable, and produced individuals resembling *brasilia*, *humilis*, *texana*, *scabra* and *"nana."* Nothing can be said concerning the fertility of the plants except that it was variable. Judging from open-pollinated capsules, the majority of the plants were more than 50 percent fertile, but whether any plants were less fertile than those of the $F_1$ generation is unknown.

*The descendants of the $F_2$ generation of the plants of experiments 1 and 2*

A considerable number of $F_2$ plants from experiments 1 and 2, *N. rustica humilis* × *N. paniculata*, were selfed, and the descendants followed for several generations. The behavior of the families of each experiment was so similar that there is no advantage in describing more than one set. In table 6, therefore, the salient features of 51 $F_3$ families arising from $F_2$ plants of experiment 2 are set forth.

The important question to be answered by these data is that of variability, and it was not an easy matter to make an estimate which would be quantitative and at the same time practical. Fifty-one families were raised, giving me a real task in trying to cover the point at issue without covering an interminable number of pages. If, for example, frequency distributions were made of a number of characters in each family, there would be an apparent accuracy in the results. But this accuracy would in truth be only apparent. What we wish to know is the variability of the families as entities. With series of data for separate characters, it would be necessary to try to keep the figures for each character in mind, and to weight these figures mentally in making a general average. Instead of doing this on paper after taking the data, therefore, it was done in the field. For this reason the figures of table 6 may be subjected to the criticism that they are distorted by the personal equation of the writer. This is true. But, on the other hand, all figures are somewhat distorted by personal equations, and I believe the data of table 6 give a better idea of the actual situation than would have been given by a more voluminous set.

The first column in the table gives the number of the $F_3$ family. In the second column is the number of plants under observation. When the plants showed a distinct type, it is listed by name in the third column. Under height, leaves, flowers and fertility, the measurements of the extremes are given in the first column. In the second column under each heading a number is given which represents the variability of the character in question on a scale of 10 where 1 represents the wonderful uniformity of the pure line of *N. paniculata* described previously and 10 represents the inordinate variability of the $F_2$ generation of experiment 2. Finally column 4 gives the general variability of each family on the same scale. This figure is frankly an estimate. But it was made in the field after the estimates had been made of the variability in height, in size and shape of leaf, in size and shape of flower and in fertility. It gives weight to these determinations, but it also considers the general characteristics of the plants in a manner that could only have been done by an observer in the field.

Owing to the small number of plants grown, families 52, 101 and 176 should be omitted. Four other families, 67, 199, 228 and 234, may also be omitted because it was so difficult to estimate their variability on a scale comparable to the others. Family 199 contained 48 plants. All but 5 of these plants were typical *N. rustica humilis* plants with a variability scarcely greater than that of the maternal great grandparent. The 5 aberrant individuals were tall plants resembling *N. rustica texana*. Omitting the *texana*-like plants the general variability of the family would stand at 2, but its rating with them included is questionable. Similar remarks might be made for the other three families. Perhaps it is better to rest our conclusions on the 44 families remaining after the omission of these seven.

Two facts stand out impressively in this array of data:

(1) The families are all rather high in fertility and low in the variation in fertility within each group. Several families showed scarcely any discernible variation in fertility and No. 131 should probably be ranked the equal of its great-grandparents. Every ovule was capable of being fertilized, and the amount of abortive pollen (3 to 5 percent) was so small one would hardly suspect the origin of the strain. (2) The uniformity of the families, visualized as relative homozygosity of each parental $F_2$ individual, is much greater than one would expect. Twenty-two families, just one-half of the total, were rated 3 or less in general variability. When it is recalled that *N. paniculata* our No. 1 in the scale is the most uniform strain ever raised in our experimental garden, whether of Nicotiana or of

TABLE 6

*Characteristics of the $F_3$ families of cross between N. rustica humilis and N. paniculata.*

| $F_3$ family number | Number of plants | Type | General variability | Height | | Leaves | | Flowers | | Fertility | | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Range | Variability | Range | Variability | Range | Variability | Range | Variability | |
| 24 | 16 | sh. *tex.* | 3 | 80–100 | 3 | 15×12–23×22 | 3 | 21×16–22×18 | 2 | 8–9 | 2 | 1 type only |
| 30 | 20 | var. | 6 | 50–110 | 7 | 9× 5–30×21 | 8 | 15×15–18×15 | 3 | 6–9 | 5 | |
| 33 | 51 | *scabra* | 2 | 110–135 | 2 | 20×15–24×18 | 2 | 18×16–19×17 | 2 | 8–9 | 2 | 1 type only |
| 49 | 49 | *texana* | 4 | 80–150 | 5 | 20×12–32×23 | 4 | 17×15–19×16 | 3 | 9–10 | 2 | 1 *bras.* type |
| 52 | 7 | var. | 6 | 70–110 | 5 | 20×14–23×16 | 3 | 20×13–21×19 | 5 | 7–9 | 4 | |
| 56 | 34 | *hum.-tex.* | 5 | 50–100 | 7 | 12×10–24×22 | 6 | 19×14–22×19 | 3 | 6–8 | 5 | |
| 64 | 30 | sh. *tex.* | 3 | 100–140 | 3 | 14× 8–25×16 | 3 | 20×16–22×15 | 2 | 5–8 | 4 | 1 type only |
| 67 | 27 | *scab.-tex.* | 4 | 110–150 | 3 | 15× 9–25×22 | 5 | 18×15–19×15 | 2 | 8–9 | 3 | 4 plants 60 cm. |
| 76 | 37 | *texana* | 4 | 70–160 | 6 | 13× 8–23×20 | 4 | 18×16–23×20 | 5 | 7–8 | 4 | Few lvs. |
| 79 | 38 | Var. | 6 | 70–150 | 6 | 15×10–25×19 | 6 | 18×13–22×18 | 5 | 7–9 | 3 | lvs. obl.-lanc. |
| 83 | 51 | *humilis* | 2 | 100–120 | 3 | 18×13–20×16 | 2 | 18×16–19×17 | 2 | 9–10 | 2 | lvs. thick |
| 85 | 51 | *scabra ?* | 2– | 130–170 | 2 | 23×16–25×18 | 1 | 18×18–19×19 | 1 | 8 | 1 | late maturing |
| 87 | 50 | *hum.-tex.* | 4 | 60–120 | 3 | 15×10–28×22 | 4 | 17×13–19×19 | 4 | 7–8 | 2 | |
| 88 | 35 | *texana* | 4 | 90–160 | 4 | 20×15–32×28 | 5 | 18×17–19×18 | 2 | 8–9 | 2 | lvs. *bras.* |
| 89 | 28 | sparsely lvd. | 2 | 105–130 | 2 | 25×20–30×22 | 2 | 18×15–21×20 | 3 | 7–8 | 2 | |
| 90 | 25 | new type | 2 | 100–140 | 2 | 25×20–31×28 | 3 | 16×15–18×17 | 2 | 7–8 | 2 | habit uniform 1 |
| 93 | 25 | *hum.-tex.* | 3+ | 80–120 | 4 | 20×12–28×24 | 4 | 16×14–20×18 | 3 | 7–8 | 2 | |
| 98 | 30 | var. *tex.* | 5 | 60–180 | 6 | 20×12–30×26 | 5 | 19×18–20×18 | 2 | 7–8 | 3 | |
| 101 | 3 | tall *tex.* | | 130 | | 26×22 | | 18×15–18×16 | | 8 | | |
| 102 | 57 | *texana* | 4 | 80–150 | 4 | 15×10–28×22 | 5 | 18×14–20×18 | 4 | 7–8 | 3 | |
| 104 | 56 | new type | 6+ | 100–150 | 5 | 20×20–30×12 | 6 | 17×16–20×19 | 3 | 7–8 | 3 | like No. 90 |
| 108 | 33 | new type | 4 | 50–150 | 5 | 20×12–28×24 | 4 | 18×17–21×20 | 4 | 7–8 | 2 | tall laterals |
| 110 | 21 | *texana* | 2 | 120–160 | 2 | 25×18–28×23 | 2 | 18×16–19×16 | 2 | 7–8 | 2 | tall type |
| 111 | 50 | tall *bras.* | 1+ | 100–120 | 1 | 24×19–28×26 | 2 | 18×15–18×16 | 1 | 8 | 1 | late |
| 114 | 44 | *texana* | 3 | 60–160 | 4 | 15×10–32×25 | 4 | 18×16–19×16 | 2 | 6–7 | 3 | |
| 115 | 52 | new type | 2 | 110–140 | 1 | 25×20 | 1 | 18×18–19×18 | 2 | 7–8 | 2 | few lvs. |

TABLE 6 (Continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 122 | 36 | *texana* | 5 | 60–150 | 6 | 17×10–30×22 | 4 | 16×12–20×16 | 5 | 8–9 | 3 | |
| 124 | 52 | tall *bras.* | 2 | 120–150 | 2 | 18×14–30×19 | 2 | 18×18–19×18 | 1 | 7–8 | 3 | |
| 131 | 40 | tall *bras.* | 1+ | 100–115 | 1 | 25×20–28×26 | 1 | 19×16 | 1 | 9 | 1 | like No. 111 |
| 139 | 50 | *texana* | 5 | 70–120 | 4 | 14×10–30×25 | 5 | 17×13–19×16 | 4 | 5–7 | 4 | |
| 145 | 50 | *scab.-tex.* | 5+ | 60–190 | 6 | 15× 8–30×24 | 6 | 18×17–19×17 | 2 | 8–9 | 2 | very large caps. |
| 146 | 35 | *humilis* | 3 | 50–90 | 3 | 15× 8–26×18 | 3 | 18×15–19×16 | 3 | 7–9 | 3 | |
| 149 | 52 | tall weak | 3 | 130–160 | 3 | 28×26–34×29 | 4 | 16×15–17×16 | 3 | 4–9 | 5 | |
| 150 | 104 | tall *bras.* | 2– | 110–150 | 2 | 25×16–27×18 | 1 | 18×14–19×15 | 1 | 8–9 | 1 | |
| 159 | 53 | *texana* | 3 | 100–160 | 3 | 25×18–28×18 | 3 | 19×15–21×16 | 3 | 8–9 | 3 | |
| 163 | 54 | sparse *tex.* | 2 | 120–180 | 3 | 24×20–26×22 | 3 | 20×16–21×17 | 2 | 7–8 | 2 | |
| 166 | 47 | *bras.-tex.* | 4 | 90–130 | 3 | 26×20–28×24 | 3 | 18×16–19×17 | 3 | 5–8 | 4 | |
| 167 | 44 | *texana* | 5 | 35–140 | 6 | 8× 6–30×26 | 6 | 14×12–20×18 | 6 | 8–9 | 2 | small type |
| 168 | 25 | elong. *hum.* | 5 | 50–120 | 6 | 20×18–28×24 | 5 | 18×15–19×15 | 4 | 8–9 | 3 | |
| 176 | 11 | 6 tall *bras.* | 4 | 110–130 | 3 | 18×16–26×22 | 4 | 18×15–19×16 | 4 | 7–9 | 3 | 5 *texana* |
| 191 | 46 | var. | 5 | 40–150 | 7 | 15× 8–28×21 | 6 | 16×14–21×20 | 6 | 8–9 | 2 | |
| 192 | 43 | var. | 6 | 60–160 | 6 | 16×15–28×26 | 7 | 16×16–18×16 | 6 | 8–9 | 3 | also lvs. 28×14 |
| 199 | 48 | *humilis* | 2? | 60–100 | 2 | 12× 8–20×15 | 2 | 18×15–19×18 | 3 | 7–8 | 4 | 5 *texana* excluded |
| 213 | 59 | *texana* | 5 | 70–190 | 6 | 17× 8–34×26 | 6 | 18×13–20×18 | 3 | 7–8 | 3 | |
| 214 | 47 | var. *hum.* | 4 | 50–130 | 5 | 10× 6–25×20 | 5 | 18×18–20×18 | 3 | 7–8 | 4 | |
| 217 | 50 | var. | 5 | 80–130 | 5 | 16×12–25×20 | 5 | 16×15–17×15 | 4 | 7–8 | 3 | lvs. sparse |
| 219 | 43 | new type | 3 | 50–110 | 5 | 15×13–25×25 | 3 | 16×16–18×17 | 2 | 8–9 | 2 | lvs. round |
| 228 | 45 | *tex.-scab.* | 3 | 110–180 | 3 | 28×20–32×25 | 2 | 18×15–20×16 | 2 | 8–9 | 2 | 3 with small lvs. |
| 234 | 42 | *tex.-scab.* | 2+ | 160–180 | 1 | 20×16–28×22 | 2 | 18×16–19×16 | 2 | 8–9 | 2 | also 2 small pl. |
| 248 | 38 | *texana* | 3 | 100–180 | 5 | 18×16–30×26 | 3 | 19×17–21×19 | 3 | 6–8 | 3 | |
| 250 | 25 | *humilis* | 3 | 60–120 | 4 | 16× 8–20×16 | 3 | 16×15–17×15 | 2 | 8–9 | 2 | |

any other genus, the fact is extremely suggestive. Our varieties *texana, jamaicensis* and *asiatica* raised from stock self-fertilized for several years and presumably inbred for a long period of time, would have to be rated nearly 3 in variability.

*Fifty percent of the $F_2$ plants of this cross, therefore, were so homozygous as to produce populations comparing favorably with those found in the so-called pure varieties of N. rustica which had been collected from various sources.*

FIGURE 14.—Very small $F_2$ segregate *N. rustica humilis* × *N. paniculata*.

Now we hope there will be no misunderstanding of the position taken here. It is not maintained that each of these 22 families was as uniform as the "pure" varieties of *N. rustica* they most resembled. But it is believed that none exceeded the "pure" strains by more than one-half point on our scale of 10. In other words both the eye and the measuring-stick told us that the variability of the "pure" strains and the segregate strains was of the same general order of magnitude.

No descendants of the 51 $F_3$ families of experiment 2 were carried further, but from the $F_3$ families of experiment 1, which, it will be recalled, were in every respect similar to those of experiment 2, several strains were carried through the $F_4$, $F_5$ and $F_6$ generations,—selfed seed being used in each instance. There were eight families in the $F_4$ generation, and several of these were split up in the $F_5$ and $F_6$ generations. This series of strains does not include the progeny of one of the *paniculata*-like individuals of the $F_2$ generation, which as we have already stated, was kept under observation. This plant produced a population resembling pure *N. paniculata* in every detail. The family showed no trace of inheritance from *N. rustica*, and was as uniform as a population of the *paniculata* great-grandparent. The individual plants seeded freely. The capsules were *full* of seed and various samples of the pollen showed it to be from 96 to 100 percent perfect, morphologically.

The remaining strains clearly belonged to the *rustica* group. No systematist would have suspected that they were the descendants of segregates arising from a cross where *N. paniculata* was used as one of the parents. Six of the strains were similar to certain *N. rustica* varieties obtained from Dr. COMES. One was clearly *N. rustica humilis*, a second was *N. rustica asiatica*, and a third was *N. rustica brasilia* in appearance. Two other strains were unlike any plants of *N. rustica* we have seen, but nevertheless would have been classified as members of that species. The first of these was a giant form, figure 13 giving a good idea of its characteristics. The second was a small type in which the plants all resembled figure 14. The remaining strains were from variable $F_3$ families resembling combinations of COMES'S varieties.

These strains were grown in order to try to answer two questions which are important in any theoretical interpretation of the results from the experiments considered as a whole. The first question concerns the variability of the families. The second question pertains to the fertility of the individuals.

The behavior of the $F_3$ families demonstrated conclusively that a much greater number of the $F_2$ plants were homozygous in the majority of their characters than one would have any right to expect had there been such recombination of hereditary factors as is usually found in varietal crosses, with no selective elimination of gametes and of zygotes. It is important then to know whether this apparent homozygosis is in truth a homozygosis of all the hereditary factors possessed by the individual, or whether recombination with the ultimate attainment of a greater degree of homozygosis is possible. Selfed individuals from the variable populations of $F_3$,

gave populations more uniform than those from which they came. This is probably what should have been expected. Again, two of the selfed populations from $F_3$ generations rating 2 in general variability, produced

FIGURE 15.—$F_2$ segregate *N. rustica humilis* × *N. paniculata* showing habit of growth not found in either grandparent.

populations in which leaf size in one case and habit of growth (this means somewhat more than mere height) in the second case were distinctly less variable than in the parental population. In no other instance could one

be certain that the 5 near-homozygous $F_3$ families tested had produced populations less variable than themselves, but it must be remembered how uniform these families were in the $F_3$ generation. Conversely, in no instance was a variability greater than that of the parental population observed.

In the case of fertility, not only was the variability of the parental population never exceeded by the progeny, but no individual of a population resulting from the selfing of a particular plant was ever less fertile than its parent. The succeeding generations always *increased* in actual fertility and in uniformity in the production of functional gametes and zygotes. In this series, only the two *paniculata*-like plants of $F_2$, already described, seemed to have perfect gamete-producing mechanisms, although by the word perfect it is not meant to exclude the possibility of an occasional aborted pollen grain. In the remainder of the $F_2$ plants, and in their descendants, many plants were found in which the ovules were all functional, but it is probable that none reached the percentage of perfect pollen grains characteristic of pure *N. paniculata* and pure *N. rustica*. In these two pure species, individuals have been found where 100 percent of the pollen grains seemed to be morphologically perfect, but in general there was from 2 percent to 3 percent of abortive grains. Whether this low percentage of aborted grains is due wholly to environment or not I am unable to say. I suspect this to be true, however, because I have found at least that high a percentage of aborted pollen grains in some individuals of every "pure" species of plant I have ever examined. The most fertile plants among the descendants of this cross, excluding the two *paniculata*-like segregates, had about 4 percent of the pollen abortive, with the one further exception of a *brasilia*-like plant where about 2 percent of the pollen was imperfect. Thus while many plants which came out of this cross compare favorably in a general way with the fertility of the two pure species that entered into it, adequate statistical proof that they equaled the pure species in fertility is not available. I believe the general experience with the cross leads one to expect the ultimate production of individuals wholly as fertile as the pure races, but definite proof has not been forthcoming.

## SUMMARY AND INTERPRETATION OF RESULTS

Those who have borne with us thus far no doubt will have been impressed with the fact that a very large amount of quantitative data has been more or less roughly lumped together in a descriptive manner. The danger in such a procedure is fully realized. It makes it difficult for the reader to

make up his mind as to whether the separate conclusions drawn are really established by adequate and critical data, or whether they are merely tentative. At the same time it must be clear to every one that the essential facts are rather broad and general in their nature, and could have been presented in no other way. I have dealt with the variability of the plants considered as units, rather than the variability of separate characters. In arriving at decisions, it was necessary to deal with characters separately, of course; but in weighting these data on the separate characters in order to accomplish the main objective,—an estimate of general variability,—personal equation necessarily enters. I believe the results justify the procedure, however, for two reasons. In the first place, if each plant character had been studied with all the refinements of modern statistical methods, it still would have been impossible to have judged the plants as units by an impersonal objective summation of these data. The subjective element would have been masked in a quantity of detail, but it would have entered into the final result. Furthermore, I believe that in such work as this the investigator who lives with his plants in the field, who uses all the quantitative data at his command, but who nevertheless brings to his aid all the somewhat intangible facts that intimate experience gives him, is able to come to a better realization of the truth than one who works over cold data obtained by others.

Our position in the matter having been made clear, let us endeavor to pick out the established facts which appear to be essential from the standpoint of genetic theory.

The two species, *N. rustica* and *N. paniculata*, species giving extremely uniform progeny when selfed, have produced reciprocal first-hybrid-generation populations almost identical with each other, and as uniform in all their characters as the parental populations themselves.

The proportion of fertile ovules formed by each $F_1$ plant was about the same; that is to say, careful manipulation would result in an average seed complement of about 3 to 4 percent of the normal. Each plant seemed to have this figure as a possibility. One might obtain no seed or any proportion of seed up to 8 percent after a pollination, but the variation was as great on one plant as another, and this variation may be taken largely as a mark of failure to provide the best conditions for fertilization plus the variation always existent in random sampling. At least one-half of these seeds will germinate.

An investigation of $F_1$ pollen formation leads me to believe that less than 0.1 percent of the possibilities of the pollen mother cells in the formation of pollen grains is ever realized, yet a study of mature anthers only

indicates that 2 to 3 percent of the pollen grains found there are functional. The histological results on pollen show that one is often seriously misled if conclusions as to what has happened during gamete formation are drawn from a casual examination of the contents of mature anthers. The pollen-germination studies showed further that apparent morphological perfection in a pollen grain is not to be construed as positive evidence of ability to function.

Taken at their face value the data on gamete formation seem to indicate that the formation of female gametes is some 40 times greater proportionately than the formation of male gametes. This result, however, can not be taken to mean that 40 functional female gametes per thousand and only 1 functional male gamete per thousand may be formed because of incompatible hereditary elements entering into the remaining non-functional gametes which do not come to maturity or do not function at maturity. A great part of this differential proportion in the formation of male and female gametes may be due wholly to factors of environment. In other words, the greater size of the cells, the larger amount of cytoplasm, or in general the better mechanism for gamete production in the ovary, make slight changes in temperature, osmotic pressure, etc., less effective in stopping gamete formation in the ovary than in the anther. There is considerable evidence for the truth of this statement in the fact that comparable percentages of seed were produced in each of the following cases, self-pollination, sib-pollination, use of pollen of either pure parental species on the flowers of the $F_1$ generation, and use of the pollen of the $F_1$ generation on the flowers of either parent.

The characters of the $F_1$ plants rather blended those of the two parents, but the resemblance to *N. rustica* was greater than to *N. paniculata*. The plants produced by back-crossing $F_1$ with each of the two parents, partook of more of the characteristics of the parent used. The variability of the back-crosses was considerably greater, statistically speaking, than that of the true $F_1$ generation, but botanically the plants were like true $F_1$ generations in that they were of one general type with much less variability than the $F_2$ generation in all characters.

The $F_2$ generation was inordinately variable. With the possible exception of certain flower relations, variation in all characters transgressed those of the grandparents markedly.

Numerous character combinations, predictable with random mating and no selective elimination of gametes or zygotes, were omitted. This statement is made after very careful consideration and a full realization of the theoretical implications. Combinations approaching *N. rustica* were much

more frequent than those approaching *N. paniculata*. It is not largely a question of salient characters being masked or of group heredity (i.e.,

FIGURE 16.—$F_2$ segregate *N. rustica* [illegible] *humilis* × *N. paniculata*. Peculiar habit of growth.

linkage), for the characters have been considered separately as well as in groups. *N. paniculata* differs from *N. rustica* in numerous characters.

Some few *paniculata*-like characters can be located in a large percentage of the $F_2$ population. But when one tries to find combinations of these characters, he comes to an *impasse*. There is a gap between *rustica*-like plants with a few traceable *paniculata* characters, until plants appear which are indistinguishable from *N. paniculata*.

Segregation of determiners for fertility occurs in $F_1$, resulting in certain $F_2$ plants giving full capsules of viable seed. With seed production taken as the criterion of fertility, the $F_2$ generation ranges from that of the $F_1$ generation to that of the pure strains. It may be that individuals inheriting less fertility than those of the $F_1$ generation appear, but there is no critical evidence that this is true. What may be taken as the facts of the case from the present evidence, is that a frequency curve of fertility on the female side runs from the 3 or 4 percent characteristic of the $F_1$ generation, to 100 percent, with a marked abmodality toward the side of high fertility. Judgment of the fertility of $F_2$ by examination of the pollen does not change this general conclusion materially. Adequate evidence of the production of perfect pollen equal to that of either parent,—98 percent maximum, shall we say,—has not been forthcoming, but plants having 96 percent of "good" pollen have been found.

Varietal distinction might be accorded almost everyone of the plants of the $F_2$ generation in the crosses where *humilis* was used as the *N. rustica* parent. The parental forms were recovered once in every 100 to 200 plants. It should be noted also that of the four plants resembling *N. paniculata* obtained, two were very fertile (one reproducing a characteristic *N. paniculata* population), and two were so sterile that no viable seed was obtained.

Reproductions of all of the named varieties of *N. rustica* were obtained in the $F_2$ of the cross with *N. paniculata*, though these forms could not all be obtained by intercrossing any two of the varieties themselves.

In the cross between *N. rustica scabra* and *N. paniculata*, about 15 percent of the $F_2$ generation consisted of delicate dwarf plants of similar type with lack of fertility seemingly correlated with delicate constitution. In the other crosses there were no noticeable correlations between fertility and other plant characters.

Observations on generations later than the $F_2$ were made only in the case of the cross between *N. rustica humilis* and *N. paniculata*. Three points stand out rather impressively: (1) As judged by $F_3$ progeny, the $F_2$ plants show a *much greater degree of homozygosity* than is the general Mendelian expectation in varietal crosses. (2) A selfed plant of the $F_2$ or of later generations produces progeny with equal or greater fertility

than itself. The few cases where a slightly less fertile daughter was obtained, probably may be attributed to physiological reasons unconnected with heredity. (3) Plants almost wholly fertile produce progeny heterozygous for many allelomorphs though the variability of the resulting population, in my experience, is always less (presumably it may be the same) than that of the population from which the parent plant was selected.

In my opinion these are the salient points which should be taken into consideration in seeking a theoretical interpretation of the experiments.

Though certain data were gathered in the years 1916, 1917 and 1919 which make the problem a little clearer, most of the facts reported here had been collected by 1915. In that year I gave a paper before the AMERICAN PHILOSOPHICAL SOCIETY which was reported in abstract in the Proceedings (**54:** 69–72). A provisional hypothesis (A) was rejected.

> "(A) Through multipolar spindles, mating of non-homologous chromosome pairs at synapsis, or other mitotic aberrations at the reduction division, the 24 chromosomes characterizing each of the two species may be distributed irregularly at gametogenesis. If some of these irregular gametes may function, the majority of the experimental data are satisfied, but there are reasons which there is not time to consider which make this scheme improbable."

Obviously any peculiar case of heredity *may* be due to a rare or even a unique distribution of the chromosomes. There may be a doubling of chromosomes as in *Oenothera gigas* and the Primula investigated by GREGORY. An extra chromosome may be found as in *Oenothera lata*. There may be the phenomenon of non-disjunction discovered by BRIDGES in Drosophila. These special cases of inheritance are important, however, only in so far as they show what peculiar results may be brought into line with the chromosome theory of heredity when it is found that the chromosomes do not behave in the usual manner. Such results make ridiculous the attitude of a certain type of scientist, who, when he obtains data he does not understand, immediately proclaims from the housetop that the whole structure of modern genetics has fallen. A special-mechanism hypothesis in interpreting the data presented here has been rejected for four reasons. (1) A special mechanism should never be invoked until a sustained effort has been made to interpret the facts by the method of chromosome distribution we have come to look upon as the *characteristic* type for both the higher animals and the higher plants. (2) Cytological observations in the hybrid plants under consideration warrant us in believing that the machine breaks down in certain cases, but that when it runs it runs in the usual manner. (3) Repeated crosses involving these species having given

similar results, and the behavior of other partially sterile species crosses that I have had the opportunity to observe (for example, the wheats) having been of the same general type, it seems likely that a rather general phenomenon is involved. (4) The facts can be given a "regular" Mendelian interpretation with a little subsidiary explanation.

The interpretation favored by the writer and submitted for consideration in 1915 was as follows:

> "(B) On the other hand the facts may be interpreted without assuming irregularities of chromosome distribution if (1) there is a group of chromosomes in each parent that cannot be replaced by chromosomes from the other parent; if (2) there is a group of chromosomes from each parent, a percentage of which may be replaced by chromosomes from the other parent, but where functional perfection of the gametes varies as their constitution approaches that of the parental forms; if (3) there are other chromosomes that have no effect on fertility and therefore can promote recombinations of characters in the progeny of fertile $F_2$ plants; if (4) a naked nucleus entering the normal cytoplasm of the egg in the immediate cross can cause changes in the cytoplasm that will affect future reduction divisions; if (5) this abnormally formed cytoplasm is not equitably distributed in the dichotomies of gametogenesis in the $F_1$ generation; if (6) it follows from (4) and (5) that $F_2$ zygotes may be formed which are less perfect in their gamete-forming mechanism than those of the $F_1$ generation; and if (7) the heterotypic division of gametogenesis does not necessarily form two cells alike in their viability."

This conception of the matter satisfied the requirements of the data. Each part was added only after careful consideration. But perhaps the statements were too concise to be understood easily. At least no comments, either favorable or adverse, were made on them by other geneticists; and a year later (1916) GOODSPEED and CLAUSEN published an interpretation of the results of some crosses between *Nicotiana sylvestris* and *N. tabacum*, in which they used a conception practically the same as my (1) and (2). This conception they called a Reaction System. The data on which they based their ideas, were briefly as follows. The argument is taken from BABCOCK and CLAUSEN (1918).

*Nicotiana sylvestris*, a species belonging to the Petuniodes section of the genus, and showing no marked variations after years of cultivation, crosses freely with *N. tabacum* varieties. In any case the $F_1$ hybrids are uniform in their characters. They are very strong vigorous plants in which the influence of *N. sylvestris* may be seen, but this influence is almost hidden by the dominance [?] of *N. tabacum* characters. Furthermore the hybrids resemble the particular variety of *N. tabacum* used in the cross.

The hybrids are almost sterile, and it is doubtful whether any seeds can be obtained by self-fertilization or sib-crosses. When *N. sylvestris* pollen is used on the hybrids, they set about 1 percent of the normal complement

of seeds. The sesqui-hybrids thus obtained show diversity of type; but about 10 percent of them resemble *N. sylvestris* very closely, are almost completely fertile, and give rise to individuals which are seemingly pure

FIGURE 17.—$F_2$ segregate *N. rustica humilis* × *N. paniculata*, showing characters not found in either grandparent.

*N. sylvestris*. Similarly a cross with *N. tabacum* pollen gives a low percentage of variable sesqui-hybrids approaching *N. tabacum* in character, and those approaching *N. tabacum* most closely are the most fertile.

In passing it may be said that the writer made this cross in 1908, and the results obtained were very similar to those of GOODSPEED and CLAUSEN.

According to the Reaction-System conception, the normal functioning of a set of hereditary factors depends upon the harmonious interrelations which the factors maintain with each other. In the $F_1$ hybrids, the resemblance to *N. tabacum* is thought to indicate a more or less complete dominance of the *N. tabacum* factors as a complete Reaction System.

"Developing the reaction system hypothesis, it would appear that, if the *N. tabacum* and *N. sylvestris* systems display a high degree of mutual incompatibility, any gamete containing elements derived from both systems would give a reaction system subject to profound disturbances incident upon the inharmonious relations set up between the *N. tabacum* and *N. sylvestris* elements. If the admixture be relatively slight, the inharmonious elements may not greatly affect the workings of the reaction system and there would result individuals showing practically the entire set of characters of one or the other parent, and such individuals would be fully fertile. A slightly greater proportion of inharmonious elements in the reaction system would result in such profound disturbances in its functioning as to produce the abnormal individuals of various kinds which make up so large a proportion of the progeny from such parentage. When the proportions of inharmonious elements in the gametes become still greater, they fail to function at all. It is upon the formation of such non-functional gametes or the attempt to produce them, that the partial sterility of the hybrid depends, and since in this particular case these form by far the greater proportion of gametes, the hybrid is very nearly completely sterile."

BABCOCK and CLAUSEN have given a diagrammatic illustration of the scheme involved here by a table showing the recombination series in the gametes of $F_1$. It is based on the fact that the haploid number of chromosomes in *N. tabacum* is 24, and on the assumption that the number in *N. sylvestris* is the same. It is reproduced here (table 7) because the number of chromosomes both in *N. rustica* and *N. paniculata* also appears to be 24, and the table will serve as a diagram in our further discussion. The gametic recombinations are given by the expansion of the expression $(1 + 1)^{24}$, the sum of the coefficients being 16,777,316. No crossing over is assumed. The diagram is not meant to be more than a possible illustration of the actual state of affairs in the germ-cells of the hybrid under discussion.

The real issue which GOODSPEED and CLAUSEN wished to call to the attention of geneticists is one to which the latter could scarcely have objection. It is one which has been discussed casually as an *obvious* genetic conception, and yet it has been the basis of criticisms directed against genetic workers by biologists who have been foolish enough to attribute to geneticists the naïve idea of a particulate inheritance in which unit characters, having no better working relation with each other than

bricks in a hod, are shuffled and combined at random. GOODSPEED and CLAUSEN deserve thanks for making it clear that geneticists have no such conception, that their real idea is that of a kind of reaction system in which the parts are parts of a smoothly working whole. Organisms may be likened to machines fitted for similar work. The same fuel, the same lubricants, the same paint may be used indiscriminately on either of two samples chosen at random without making much difference in the working

TABLE 7

*Recombination series of gametes of $F_1$ of Nicotiana species crosses. (After Babcock and Clausen).*

| CONDITION OF GAMETES | *Tabacum: sylvestris* CHROMOSOMES | PROPORTIONATE NUMBER OF GAMETES | PROGENY WHEN POLLINATED WITH *N. tabacum* | PROGENY WHEN POLLINATED WITH *N. sylvestris* |
|---|---|---|---|---|
| Functional | 24:0 | 1 | Plants resembling the *N. tabacum* parent and of various degrees of fertility | Plants resembling the $F_1$ and abnormal plants, but all nearly completely sterile |
| | 23:1 | 24 | | |
| | 22:2 | 276 | | |
| | 21:3 | 2,024 | | |
| | 20:4 | 10,626 | | |
| | 19:5 | 42,504 | | |
| Non-functional | 18:6 | 134,596 | No viable seeds | No viable seeds |
| | 17:7 | 346,504 | | |
| | 16:8 | 736,321 | | |
| | 15:9 | 1,307,504 | | |
| | 14:10 | 1,961,256 | | |
| | 13:11 | 2,496,144 | | |
| | 12:12 | 2,705,456 | | |
| | 11:13 | 2,496,144 | | |
| | 10:14 | 1,961,256 | | |
| | 9:15 | 1,307,504 | | |
| | 8:16 | 736,321 | | |
| | 7:17 | 346,504 | | |
| | 6:18 | 134,596 | | |
| Functional | 5:19 | 42,504 | Plants resembling the $F_1$ hybrid and nearly completely sterile | Abnormal, infertile plants and fertile plants closely resembling *N. sylvestris* |
| | 4:20 | 10,626 | | |
| | 3:21 | 2,024 | | |
| | 2:22 | 276 | | |
| | 1:23 | 24 | | |
| | 0:24 | 1 | | |

efficiency. But let one try to interchange carburetor parts, engine parts, or driving mechanisms, and it becomes a question of whether these parts are closely enough related to be exchanged without upsetting the mechanism in its entirety. Just one misfit of an essential part, and the machine no longer goes.

With this idea we are wholly in agreement, but we wish to go a little further and try to picture the reproductive mechanisms of the plants

involved, in such a manner as to satisfy the known facts of our investigations.

In the first place let us consider the $F_1$ generation of the cross between *N. rustica humilis* and *N. paniculata*. The two parental strains having shown a very high degree of uniformity, one must assume that each is homozygous for practically all hereditary factors, that every gamete of each type has the same constitution, and that all of the $F_1$ zygotes are therefore alike. These assumptions fit in with the fact that the $F_1$ population showed no more variability than the parental populations. Again, one must assume that the cytoplasm of the germ-cells of the parents has little effect on the actual characters of the $F_1$ generation, because nearly all, and possibly all of the cytoplasm is furnished by the maternal parent, and the reciprocal $F_1$ generations are to all intents and purposes identical. But there is no reason why one cannot suppose that the cytoplasm helps lubricate the machinery of the reduction division, so to speak.

Turning now to the gamete production of the $F_1$ generation, let us glance at table 7. Manifestly the actual production of gametes falls far short of the theoretical production possible when none is aborted. But do the pedigree-culture facts and the cytological observations give us any reason for concluding how and why this abortion occurs? Let us see. Clearly the obvious suggestion is the one we have given in B (2) to the effect that there are "chromosomes in each parent that cannot be replaced by chromosomes from the other parent." As GOODSPEED and CLAUSEN would express it, certain chromosome combinations will fail to function because the reaction system has been upset. The simplest diagram of the situation is that suggested in table 7 where the 6 chromosome combinations at each extreme carrying 19 to 24 chromosomes of one parent and 1 to 6 chromosomes of the other parent are the only ones functioning, thus providing for about 7 functional gametes per thousand. The difficulty with this diagram is that it is too simple. The proportion of functional gametes is at least 30 per thousand, judging from the fertile ovules. In individual capsules it ran up to about 100 per thousand, but this high figure may have been a distorted result caused by insufficient samples. With due regard for the theory of random sampling, however, 30 per thousand is somewhere near the maximum limit. Of course the proportion of functional gametes appeared to be much less, but part of the sterility exhibited there may have been due to causes that had little to do with selection according to hereditary constitution. It seems to me that this is a fair deduction from the fact that reciprocal back-crosses produced about the same amount of seed. To fit these facts, therefore, one must suppose that

from 20 to 24 chromosomes of one of the parents must be present in the functional gametes produced by the $F_1$ generation.

Now the above assumptions will account for the sterility found in the $F_1$ generation, for the variability exhibited in the $F_2$ generation, and the frequent appearance of types like the grandparents which appear there. If a differential compatibility is assumed at the ends of the table, i.e., if more of the *paniculata* chromosomes must be present on the one hand than of the *rustica* chromosomes on the other hand, in order to produce functional gametes, the *rustica*-like appearance of all but a few $F_2$ individuals is accounted for. In fact one should assume functionality in the first case only when either 23 or 24 chromosomes of *paniculata* are present. But with these simple assumptions one does not fully satisfy the following conditions found in the $F_2$ generation, viz., the appearance of plants identical with other *rustica* varieties, and the appearance of homozygous individuals with great frequency.

These two phenomena seem to be related phenomena of considerable significance. Do we not have here an addition to the steadily accumulating evidence on simple Mendelian unit characters, that changes in the germ-plasm are largely changes in organization which affect only an end result? Here, for example, one finds in *N. rustica humilis* a variety capable of producing plants having certain definite characters. But when crossed with *N. paniculata*, the other well known varieties of *N. rustica* appear in the $F_2$ generation. Is this not an indication that in the germplasm of *N. rustica humilis* there exist the potentialities of producing all of the other varieties waiting only the chance for a particular reorganization which comes when the cross with *N. paniculata* is made? To the writer's mind the simplest interpretation of the appearance of these types and their appearance in relatively homozygous conditions, is that *N. rustica* and *N. paniculata* in their evolutionary divergence from each other have not varied in all of their chromosomes. One might suppose, for example, that among the 24 chromosomes of the two species, 12 are identical in composition, and therefore in function; 6 others differ in composition (unit factors) very slightly; while considerable evolutionary divergence has come about in the remainder. It is not necessary to draw a concrete picture of the results to be expected from these assumptions. It is clear that any degree of gametic sterility necessary to satisfy the experimental evidence can be obtained, that one can obtain many different types in $F_2$ and that many of these types will be to a high degree homozygous.

With these facts in mind, the postulates of B (1), B (2) and B (3) will stand, provided that in B (3) some of the chromosomes having no effect on

fertility are assumed to be identical in the two species. The postulates B (4) to B (7) were made to interpret what at that time was thought to be a fact,—recombination which gave plants of the $F_2$ generation *less* fertile than those of the $F_1$ generation. There is a grave difficulty in the way of interpreting such behavior on the assumption that difference in genetic constitution is the basic cause of sterility. The greatest degree of genetic difference, heterogeneity if you will, exists in the $F_1$ generation. Recombinations, whether by the shuffling of whole chromosomes, or by crossing over, increases homozygosity. *A less degree of genetic difference in the $F_2$ generation*, therefore, cannot be *the cause of greater sterility* than was exhibited by the $F_1$ generation. For these reasons, we interpreted the matter by assuming some sort of a mechanical control of the process of reduction by the cytoplasm, a cytoplasmic lubrication of the process, so to speak. Building on this foundation, increased sterility in the $F_2$ generation can be explained very clearly by the four postulates B(4) to B(7). Under our original presumption of fact, all of them are necessary, and none is on its face improbable. But since the evidence of lessened fertility in any of the individuals making up the $F_2$ generation is inadequate, and the proportion of individuals where a possible lessened fertility exists is very small, these hypotheses may be discarded as unnecessary. Lessened fertility in $F_2$, if it exists at all, may be the result of special conditions unconnected with our assumption of the general correlation of sterility with a high degree of genetic heterogeneity. It can be interpreted by the assumption that special factor combinations enhance sterility. For example chromosome pair $\frac{AB}{AB}$ in one parent and chromosome pair $\frac{ab}{ab}$ in the other parent may function in such a way as to provide for fertility. The $F_1$ combination $\frac{AB}{ab}$ may also be fertile. But the combinations $\frac{Ab}{Ab}$ and $\frac{aB}{aB}$, possible after the formation of crossover gametes, may be sterile.

If we are to make a general summary of the results of this work, then, it will be an extension of certain conclusions reached by EAST and HAYES in 1912. In this paper a series of crosses between various Nicotiana species were discussed from the standpoint of their behavior as first-generation hybrids. Vigor of heterosis was seen to accompany hybridization far beyond that point of difference between the species entering into the combination which produced sterility. Then came a degree of difference which accompanied a sudden drop in vigor to a point below that of either parental type. The conclusion was that at a certain point differentiation in gametic

structure is so great that the reduction division with the formation of all the possible types of gametes does not take place in the normal manner. At this point of differentiation, however, ontogeny may go on normally. With a still greater differentiation in gametic structure even ontogenetic cell division does not proceed in the usual manner, and a marked lowering of vigor occurs. Accompanying a constant increase in the constitutional differences exhibited by the species entering into the hybridization, as assumed to exist from the behavior of the crosses, comes a constantly greater deficiency in ability to go through the life cycle, the last stage before total incompatibility is reached being the production of seeds having a partly developed endosperm but no embryo.

These experiments carry one of the ideas expressed at that time a little further. Numerous matings were made between two species in which differentiation had gone just far enough to cause the great majority of the gametes to cease to function; yet in all the behavior of the pedigree cultures and in all of the cytological observations, the evidence is that inheritance proceeds in a normal Mendelian manner. With similar gametes uniting to produce an $F_1$ generation, great uniformity results. Ontogenetic division has proceeded regularly in spite of the unlike constitutions of the two haploid sets of chromosomes possessed by each nucleus. When gametogenesis occurs in the immature germ-cells of the hybrids, the evidence is that it also takes place in a wholly normal manner, but that only a part of the resulting gametes are functional. What is more natural than to suppose that the gametes eliminated are those in which chance has thrown together such a nearly balanced lot of chromatin of different constitution that proper reorganization in preparation for their essential gametic functions is impossible? And the evidence of the pedigree cultures bears out this view.

Other inductions may be made from the behavior of the cultures, however, without drawing upon the imagination. In the first place, it is clear that if the assumption as to the cause of sterility be granted, more chromosomes of *N. paniculata* are necessary for proper functioning of the germ-cells when they are in the majority than when those of *N. rustica* provide the excess. Secondly, the variability of the $F_2$ generation, the peculiar types produced by a small number of $F_2$ seeds, and the behavior of the $F_3$ generations produced by self-fertilized $F_2$ individuals, make it seem probable that certain heterozygous combinations are formed but are eliminated as zygotes. It may be asked why, if the $F_1$ individuals go through their development normally when they must of necessity possess the greatest degree of heterozygosity, $F_2$ individuals should be eliminated? The ques-

tion is pertinent, but not unanswerable. The gametes of the parental strains are formed under natural conditions. They are perfect gametes. When fertilization occurs ontogeny proceeds in an orderly manner, and abnormal conditions are manifested only at gametogenesis. At gametogenesis some of the gametes expected are formed, others are eliminated. Does it not seem probable then, that a certain proportion of those functional gametes formed by the $F_1$ plants are still so abnormal in structure that when fertilization occurs development is difficult? Now it seems wholly reasonable to assume that difficulty of development is in this instance correlated with heterosis, i.e., with unlike chromosomes, just as difficulty in gametogenesis is similarly correlated in the $F_1$ generation.

It is further probable from the ratios obtained, that a considerable percentage of the chromosomes of the two species have not been differentiated in constitution, or have been differentiated very slightly. In fact this last assumption appears to be the simplest way of accounting for the occurrence of the duplicates of varieties *brasilia*, *scabra* and *texana*, in the cross with *N. paniculata* where only *N. rustica humilis* was used.

Finally, there is a rather peculiar circumstance for which to account in the comparatively low variability of the back-crosses of the $F_1$ generation with either parent. In varietal crosses similar matings give a less degree of variability than a true $F_2$ generation, but the extreme variability of type of the $F_1$ gametes is unquestionably brought out. Here the variability, though considerably greater than that of the true $F_1$ generation, is not such as would appear to be possible if all of the $F_1$ gametes functioning in the production of a true $F_2$ generation were to unite with the gametes of the pure species and produce viable zygotes. Confessedly, this phenomenon is somewhat of a puzzle. I can see in it only a further elimination of the extreme gametes of the $F_1$, but just why such a peculiar thing should occur, is not clear. Perhaps our observations are distorted in that we had become accustomed to an inordinate variability in the combinations of gametes forming the $F_2$ generations, and had expected a greater variability than was proper when a series of like gametes mated with those of the $F_1$ generation. Possibly a variability standing half-way between that of the $F_1$ generation and the $F_2$ generation was not to have been expected. Certainly the variability exhibited by the back-crosses was more that of an $F_1$ generation than one would suspect would be the midpoint between the $F_1$ generation and the $F_2$ generation. But this may simply be an erroneous idea.

Such, then, are the theoretical conclusions to be drawn from the data. But there is also a conclusion of practical significance which ought not to

be neglected. Not only these experiments but other critical experiments on plants indicate that the greatest opportunity for plant improvement and possibly for animal improvement as well, lies in crossing together types which have become as far differentiated from each other as is possible without producing complete sterility in the hybrids. When species differing in a large number of allelomorphs can be crossed, and the resulting hybrids are completely fertile, there is a wonderful chance for desirable recombinations occurring in the $F_2$ generation. In cases of this kind that we have studied, however, the appearance of the $F_2$ individuals and their behavior on further breeding indicate a large number of *minor* variations in the chromosomes. The variability of $F_2$ is great, it is true, but recombination of a great number of factors having a minor effect on the morphology or the physiology of the individual, gives a graded series difficult to distinguish the one from the other. Opportunity for selection is there, but with so many individuals massed in the intermediate classes, practical use of selection is rather difficult. On the other hand, when two species are still further apart in their constitution, so far indeed that partial sterility becomes characteristic of the $F_1$ individual, the chromosome differences produce marked results even though the great majority of the gametes and zygotes is eliminated. That is to say, the greater the differentiation of the protoplasm the greater the chance for extreme differentiation in the individuals which do pass through the selective sieve that eliminates so many. Therefore one may assume that the greatest opportunity for obtaining new and desirable domestic forms is by crossing types as different from each other as is possible with some degree of fertility in the $F_1$ generation.

Again, we believe that just those families and genera that have varied widely in nature, yet have retained that necessary physiological compatibility which allows them to cross and to produce more or less fertile hybrids, are the ones—the only ones—that have been *really important* as domestic plants and animals. Some of the genetic evidence, and most of the historical evidence relating to the important cultivated species indicates a polyphyletic orgin. Such critical data as we have, therefore, indicate that it was possible to domesticate, to improve and to use the forms man has utilized, just because nature had prevented too great sexual incompatibility from accompanying the physical differentiation that had arisen in certain genera through natural selection. In fact we believe this conclusion has the dignity of a natural law; viz., the desirability of a type for domestication varies directly with the amount of physical variation and inversely with the amount of sexual incompatibility accompanying such variation. We

believe that plant breeders and animal breeders should emphasize this more and more in a practical way, that they should never lose sight of the fact that conscious selection of valuable types of organisms is a useful tool only when there is material for selection. And crossing, wide crossing, furnishes the greatest opportunity to obtain this material.

It has been the writer's experience that the evidence as to the polyphyletic origin of the great groups of domesticated plants and animals, based on historical and anthropological grounds, is not fully appreciated. When this evidence is compared with the genetic evidence of compatibility at hand, there is really an extremely good case *a priori* for asking the government to undertake practical plant breeding and animal breeding through hybridization on a much greater scale than is going on at present.

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# A FURTHER REPORT ON SPECIES CROSSES IN BIRDS

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## INTRODUCTION

In 1915, several controlled crosses with ducks and pheasants were described. The data for the present paper are in part from a continuation of these experiments, and in part from new crosses carried on during 1916 and 1917. In a sense it is a final report, since the exigencies of the war caused the abandonment of the investigations in 1917.

Analysis of the material, taken as a whole, presents a picture which may be of some interest. At least it brings a certain amount of order out of an apparent chaos, presumably caused by sporadic reports of numerous hybrids between different species of birds in which the number of individuals under observation has been insufficient for arriving at any proper conception of the mechanism of heredity. The impression appears to have been created that there is seldom anything approaching a Mendelian phenomenon in species-mating among birds. But definite types of segregation do occur, and these types are dependent upon the degree of relationship of the genetic constitution, as I shall hope to show here.

## MALLARD DUCK × FLORIDA DUCK

In 1915 the following cross was produced. The male parent was *Anas fulvigula maculosa*, a resident of the Gulf Coast of Louisiana and Texas, and a very poorly marked subspecies of the Florida Duck (*Anas fulvigula*) from which it differs by only one or two doubtful characters. The female parent was the Mallard. This cross was undertaken chiefly with the idea of testing the systematic position of the Florida Duck as compared with the common Black Duck (*Anas rubripes*), as seen in their hybrids with the Mallard.

*Anas fulvigula* (the subspecific name is dropped for convenience) resembles a light-colored Black Duck. The sexes are the same, except for the color of the bills, both resembling the female Mallard very closely. The only marked difference is that the white speculum band of the latter is not present. From the genetic standpoint the Florida Duck may be regarded as a Mallard with the male plumage (secondary sex characters) dropped out.

The first-generation hybrids were very different from the Mallard × Black-Duck combination, being much lighter-colored and more Mallard-like, and showing a slightly greater range of variation than is usual in a first-generation cross (figure 1). 17 males and 14 females were reared.

The next year (1916) an $F_2$ generation of 23 males was produced (see figure 2); and in 1917, 15 more were reared,—the number of females being approximately the same. The result is shown better in the photograph than by description, but it is so striking as compared with most other series of second-generation species hybrids, that it deserves special mention. These 38 males, when arranged in a linear series, present birds approaching the Mallard parent at one end, birds with solid-green heads, white neck-rings, chestnut breasts, and light-colored, vermiculated abdomens; while at the other end are birds with almost no male secondary sex characters, plain brown types, looking almost exactly like $F_1$ females, and to be distinguished from females only by the color of their bills, by their voices, and by the presence of testes. Between these extremes is a continuous series showing every gradation, including a few mixed types, such as birds with very Mallard-like body-coloring and small white neck-ring, but with female-like, brown heads.

Taking the body regions separately, there is the following result in the $F_2$ generation.

*Heads.* Solid green to solid brown. There were 2 with solid green heads, 5 or 6 with mottled heads and the remainder with little or no green.

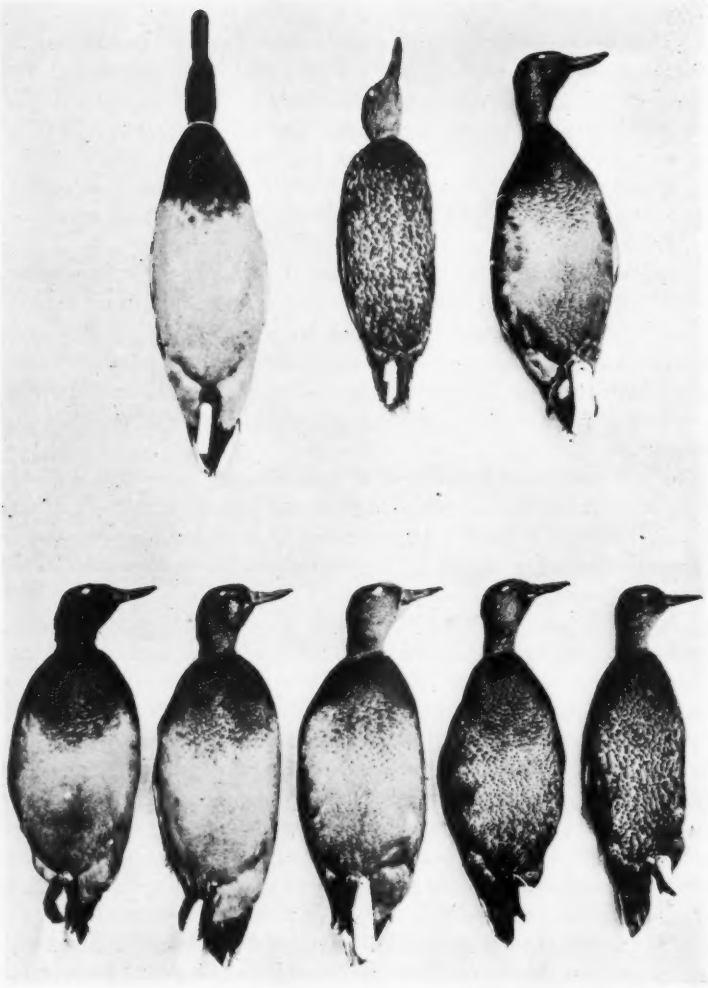

FIGURE 1.—Mallard Duck × Florida Duck. All males. Upper row, left to right: Mallard, Florida, $F_1$ hybrid. Lower row, selected $F_2$ types, left to right: No. 1, extreme Mallard type; No. 2, Mallard type with spotted head; No. 3, $F_1$ breast and abdomen, but with brown head; No. 4, approaching Florida type; No. 5, extreme Florida type showing scarcely any male secondary sex characters.

The birds with the greatest amount of green on the head, as a rule, were the most Mallard-like.

*Bills.* No great difference in series.

*Neck ring.* A trace in 4 specimens, absent in all others.

*Breast.* From almost solid chestnut with a few black spots, through mottled chestnut, to pure female type of breast color (mottled brown).

*Abdomen.* Unspotted and almost pure Mallard type in 4 specimens, through many intermediate types, to pure female type.

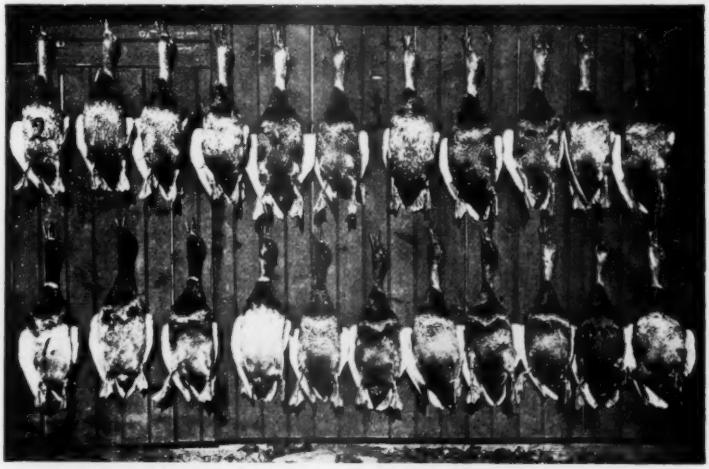

FIGURE 2.—Mallard Duck × Florida Duck. Male $F_2$'s. 1, extreme Mallard variate; 2, extreme Florida variate. Arranged in series, upper row left to right, lower row right to left.

*Under-tail coverts.* All more or less of Mallard type, that is to say, black. The amount of black varies directly with Mallard-like appearance. Traces of the dark under-tail coverts persist in males that have lost practically every other trace of secondary sex characters.

*White anterior wing-bars.* More marked in Mallard type and always absent in the female or the neutral types.

*Rump.* In series the more Mallard-like birds have darker rumps. This is not universal. There is one intermediate individual with a very black rump.

*Color of legs.* The markedly Mallard types have brighter, more orange-colored legs than those at the other end of the series.

*Back and scapulars.* Rather dark and uniform throughout the series, but as one approaches the intermediate and the female type of male, the ends of the feathers become edged with lighter colors.

*Tails.* The lighter, brownish tails (upper surface) appear as a rule at the Mallard end of the series, but not in every case. The upturned sex feathers (central tail feathers) are more pronounced in Mallard-like individuals.

The females show about the same variation that one finds among the specimens of pure-Mallard females.

It is apparent then that in this species cross we have a nearer approach to orthodox Mendelism than in any other thus far reported among birds. Out of 38 males two are nearly pure Mallard types, and three almost pure female types, these latter closely resembling the Florida-Duck parent.

A back-cross made in 1917 between a female Mallard and an $F_1$ Mallard $\times$ Florida resulted in a series of 13 males which were very uniform and extremely close to Mallard. They do not show the variation characteristic of the $F_2$ series, in fact they present very little more variation than one sees in a normal $F_1$. All except three are birds with solid-green heads, white neck-rings, and light-colored abdomens. Two approach the $F_1$ type and one other has a partly green head. The white neck-ring is never complete as in pure Mallard, but broken on the dorsum. There are no "female" types like those in the $F_2$ series. The females of this back-cross, 23 in number, show some specimens impossible to distinguish from pure Mallard.

### MALLARD DUCK $\times$ BLACK DUCK

A cross between the Mallard and the Black Duck was described in a former paper (PHILLIPS 1915, p. 76). The cross was again made in 1916 and 1917, yielding 7 more $F_2$ males and 4 females. Thus there are 23 male and 27 female $F_2$ individuals from this combination. The extreme Mallard variates of this series, males 174 and 2262, do not approach the Mallard variates of the Mallard $\times$ Florida-Duck $F_2$ series; neither do the variates towards the Black Duck approach that species as closely as in the other cross. In other words, the Mallard-Black-Duck crosses do not show anything like as close an approximation to Mendelian behavior as do the Mallard-Florida crosses. The same thing is true of the Mallard-Australian crosses. Here even less segregation is seen than in the Mallard $\times$ Black-Duck crosses. There was no approach to the Mallard types in the Australian crosses, a result shown in my former paper (PHILLIPS 1915). The scheme therefore is as follows:

*Results*

| | |
|---|---|
| Mallard × Florida Duck | 38 $F_2$ males obtained. 2 nearly pure Mallard types, 3 nearly pure Florida types. Back-crosses to Mallard females bring out many nearly pure Mallards. |
| Mallard × Black Duck | 23 $F_2$ males obtained. 2 most extreme Mallard variates lack solid-green head, white neck-ring, and light-colored, vermiculated abdomen. Back crosses to Mallard females show no pure Mallard types. |
| Mallard × Australian Duck | 15 $F_2$ males obtained. Very slight segregation. Most extreme Mallard type scarcely differs from $F_1$ individuals. Back-cross to Australian female was the only one made, so not directly comparable with the other back-crosses. |

### MALLARD DUCK × PINTAIL DUCK

For comparison with the crosses just described may be mentioned the Mallard-Pintail crosses reported in 1915. It was then shown that in this mating between the two genera, Dafila and Anas, the smallest possible amount of segregation was found, both in the straight $F_2$ generation and in back-crosses with the Pintail male. The character complexes in the parent species are here wholly unrelated, the color patterns and the colors themselves being very different, and the hybrids show a resulting tendency to stability. This experiment has not been continued since 1915.

### GOLD PHEASANT × LADY AMHERST PHEASANT

In the paper mentioned above (PHILLIPS 1915), I described part of a series of crosses between the two pheasant species known as Gold and Lady Amherst, and at that time called attention to a supposed difference in the $F_2$ hybrids of the reciprocal crosses. In order to test this matter further I repeated the reciprocal crosses in 1914; but this time used only stocks which had been tested for several generations in my own pens. These hybrids were carried to the $F_2$ generation in 1915, the males reaching maturity in 1916. There were thus 12 additional males available for study, and these show without question that there is no difference in the reciprocal hybrids. The question then comes up, what produced the opposite result the first time the cross was made? The explanation of this seems to me to be as follows. It was found that in the $F_2$ individuals of 1911–1914, the Gold × Amherst mating produced second-generation hybrids with considerably more Gold Pheasant characters than did the Amherst × Gold mating. I suggested then (PHILLIPS 1915, p. 99), that the

male Amherst used might have been a bird of hybrid origin, and that latent Gold characters influenced the offspring and produced the different $F_2$'s of the reciprocal hybrids. I still think this is the correct explanation; and this last repetition of the experiment fully bears out the conclusion that there is no evidence of sex-linkage.

### *Back-crosses*

Besides the crosses enumerated above the following 8 matings were carried out between Gold-and-Amherst hybrids:

If we designate Gold female × Amherst male as cross "C," and Amherst female × Gold male as cross "H," the scheme will be as follows:

| | |
|---|---|
| Gold female × C male | Gold female × H male |
| C female × Gold male | H female × Gold male |
| Amherst female × C male | Amherst female × H male |
| C female × Amherst male | H female × Amherst male |

Males from all these matings were finally secured and 41 were brought to adult plumage (14–16 months of age). Twenty-one specimens were preserved and careful notes made on all those discarded. All extreme variates were preserved.

Sixty-eight females were produced in the course of these eight matings, and sixteen were preserved.

It is sufficient to say here that all of these hybrids show exactly what would be expected from back-crosses of the types made. All four of the back-crosses with pure Gold stock are alike, and show a plumage intermediate between $F_1$ hybrids and the pure Gold parent. We need not therefore consider these eight matings again. There is no evidence of sex-linkage.

### *Segregation*

We come now to the question of segregation. These two species of the genus Chrysolophus present a number of sharply defined and vividly contrasted characters which could not be better for a genetic study. Those characters which present the widest contrasts may be described as follows, the male sex alone being considered, as the females in the two species are almost identical (figures 3 and 4):

| *Character* | *Male Gold* | *Male Amherst* |
|---|---|---|
| Top of head | Golden yellow | Bronze green |
| Crest | Golden yellow | Blood red |
| Mantle | Orange, banded with black | White, banded with black |
| Under parts | Uniform scarlet | Chest dark green, abdomen pure white |
| Middle tail feathers | Black with rounded spots of brown | White with black bars and lines |

It is apparent that from the genetic point of view—assuming that we are dealing with closely related sets of characters—there is here an excellent chance for the reappearance of new types in $F_2$; but with the number at hand such is not the case.

The $F_1$ hybrid males show some green on the breast, and nearly always have a yellow band, or at least a yellow area, at the point of junction of the Amherst green breast and white abdomen. This character, however, is somewhat variable. In one specimen the yellow "break" consists of a few yellow feathers on the sides of the breast, in another the band is 3.7 cm broad all across the lower surface. These represent the two extreme $F_1$ variates. All other characters are intermediate and extremely constant.

The $F_2$ generation (excluding that from the presumably hybrid Amherst male) is merely a repetition of the $F_1$, but with the extreme variates slightly extended. The nearest approach to the Gold parent has uniform scarlet underparts, mantle dirty yellow and black, head and crest pinkish straw-color, and tail vermiculated, not barred. At the other end of the scale, the most Amherst-like bird is characterized by a yellow band across the chest 5 cm wide at the sides, the scarlet of the underparts not so deep in tone, the mantle pure white and black, and the central tail feathers yellowish-white, irregularly barred and mottled with black. Segregation is manifested far less than in the Mallard × Florida-Duck cross.

### *Segregation in back-crosses of Amherst and Gold Pheasant*

Let us next consider results obtained from mating pure Amherst with $F_1$ (the four matings designated above). This series of 18 males shows some rather marked variates. No. 1765 has the lower parts nearly all red, and separated from the green breast by a whitish area. The tail is very clearly barred, the occiput and the crest deep scarlet (see figures 3 and 4). No. 1502 is nearly pure Amherst underneath, with only a little red on the lower part of the abdomen. The top of the head is nearly all green and the crest is blood red. The middle tail feathers are well barred, but not so white as in the last specimen (see figures 3 and 4).

Similarly we may describe the results obtained from mating pure Gold with $F_1$ (the four matings designated above). This series of 23 males shows a marked variation in the crest, from light straw-color in No. 999 to light scarlet in No. 1160. In No. 640 the ground color of the mantle is orange, and in two others it is nearly white. The scapular feathers show considerable variation. The lower parts are all a uniform red except

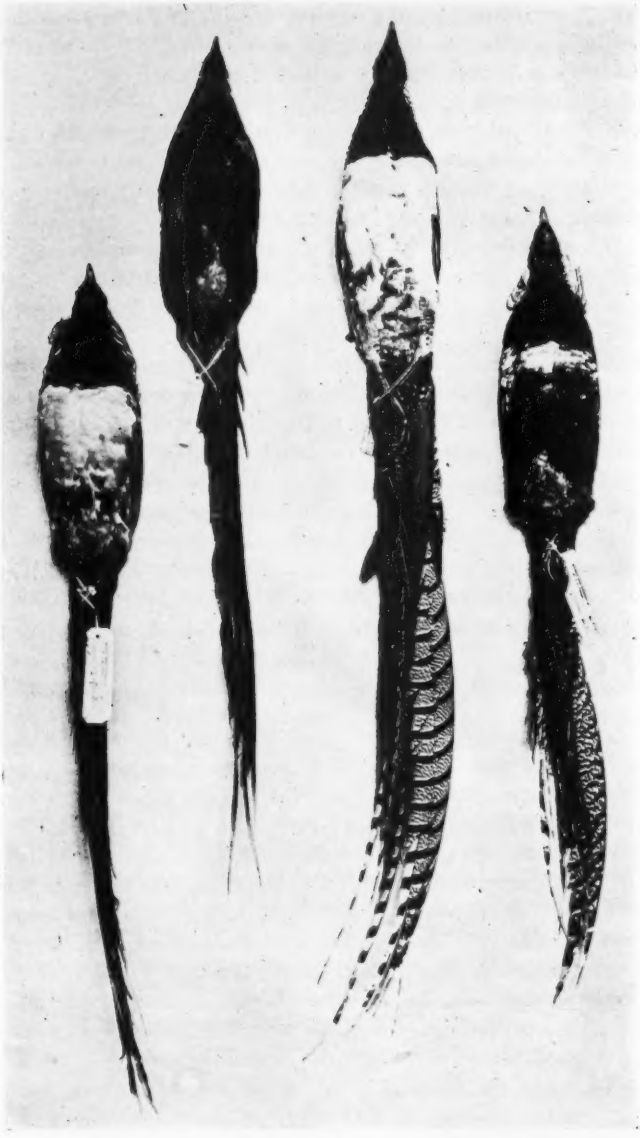

FIGURE 3.—Gold Pheasant × Lady Amherst Pheasant. All males. Upper row, left Gold; right Lady Amherst; lower row, left No. 1502, $F_1$ × Lady Amherst showing no Gold characters on lower parts; right No. 1765 of same cross showing posterior parts to white band pure red, like Gold parent.

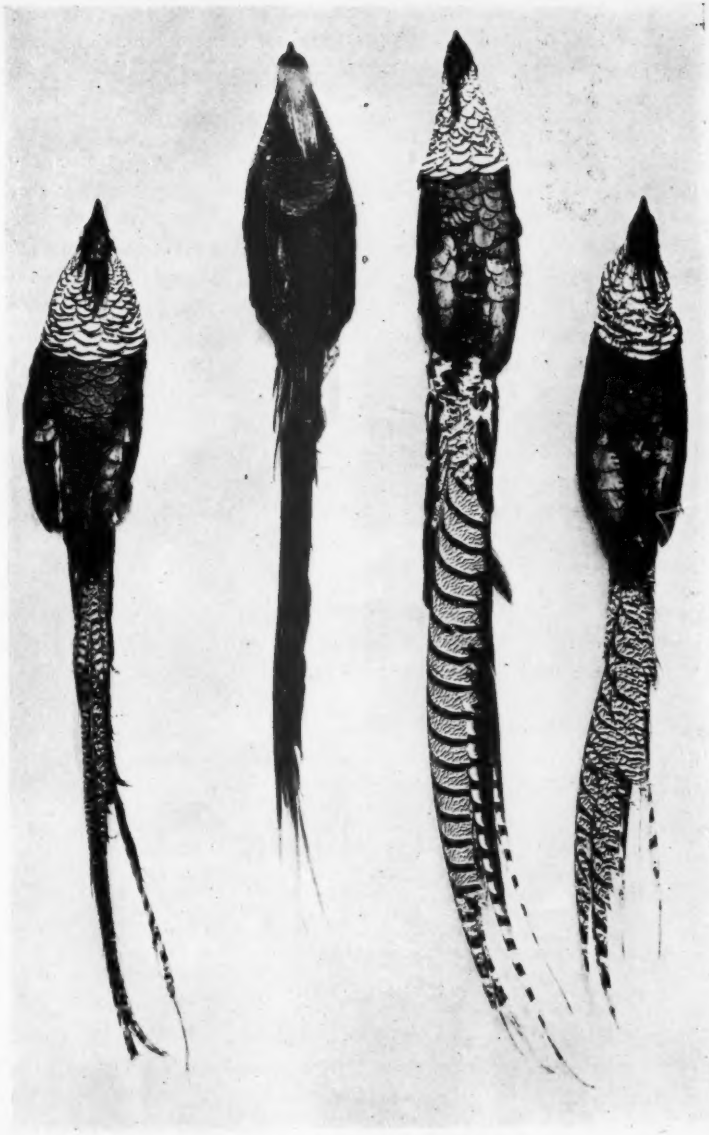

FIGURE 4.—Dorsal view of birds of figure 3.

for a few green feathers on the upper breast in a few cases. Only in one specimen, No. 1160, is there a considerable green chest-band, and in several others there are no green feathers at all.

*Linkage of character-complexes in extreme variates*

In a general way, through this entire series of back-cross hybrids, those specimens showing one character varying towards the Amherst parent, will also show several others. The same is true of variates towards the Gold parent. In other words, the character-complexes for different tracts of the plumage are more or less linked. This linkage is not, however, absolute. For example, the three-fourths-Amherst hybrid No. 1765, though having a very red (Golden character) breast, has central tail feathers even more clearly barred and Amherst-like than the extreme Amherst variate No. 1502 (see figure 3).

In the three-fourths Golden series, No. 999, with the palest head and crest, does not possess the most deeply colored (Golden) mantle, the latter being found on specimen No. 806, a bird having a pink head and crest. Thus the Golden characters are not completely linked any more than are the Amherst.

*Fertility*

There is no direct evidence of infertility in this cross. The inbred $F_2$ generation is very feeble and difficult to rear. The first cross, on the other hand, is more vigorous than either parent.

*Sex of hybrids*

In so far as records were kept the sex proportions are as follows:

| GENERATION | MALES | FEMALES |
|---|---|---|
| $F_1$ | 31 | 40 |
| $F_2$ | 28 | 27 |
| Back-crosses | 41 | 64 |
| Total | 100 | 131 |

BLACK KALIJ PHEASANT × SILVER PHEASANT

The Black Kalij Pheasant, *Genaeus melanotus* was crossed with the well known Silver Pheasant, *Genaeus nycthemerus*, in 1915 (cross F, 1915), the latter being used as the male parent. In 1916 and 1917, eight $F_2$ males were reared. These were kept until full plumage appeared at about eighteen months of age, and then killed for specimens.

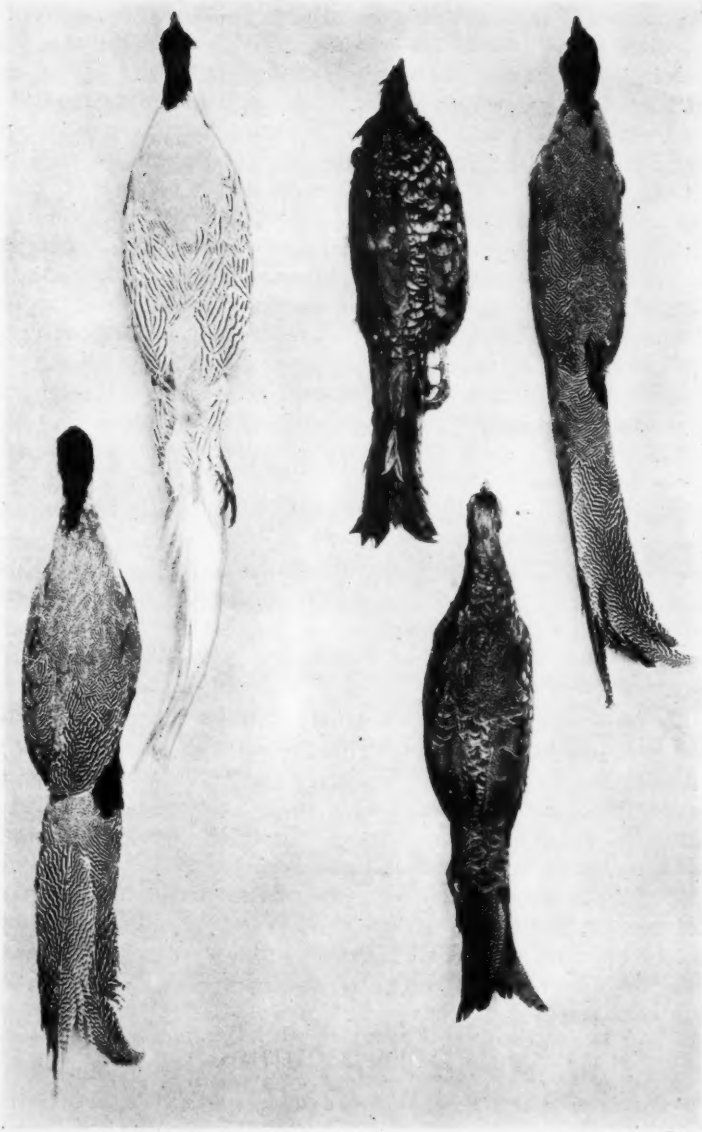

FIGURE 5.—Silver Pheasant × Black Kalij Pheasant. All males. Upper row, left to right: No. 1, Silver, No. 2, Black Kalij, No. 3, $F_1$ hybrid. Lower row, left to right: No. 1 extreme Silver variate of $F_2$, No. 2, extreme Black Kalij variate of $F_2$.

The parents of this cross differ remarkably in color, although there is no apparent infertility in the $F_1$ individuals. With the exception of head and crest, the male Silver has the whole upper surface white, with narrow black concentric lines on each feather. The top of head, crest and underparts are black. The male Black Kalij Pheasant is entirely black with a purplish gloss. The females of the two species are not markedly different.

The first cross is intermediate. The birds resemble the Silver Pheasant, though darker and more heavily barred on the upper surface, and with enough white to give them a distinct silver-gray appearance (figure 5). The eight $F_2$ males are very interesting, as they vary from the $F_1$ type to a bird (No. 1219) almost black all over, particularly so marked on mantle, wings and tail. A vermiculated appearance is seen on back rump and secondaries, but this individual might easily be taken for three-fourths pure *melanotus* (see figure 5).

### SILVER PHEASANT × SWINHOE PHEASANT

Swinhoe's Pheasant was crossed with Silver Pheasant in 1914. These hybrids were partially sterile, and only 2 $F_2$ males were reared to maturity. These two individuals are extremely different in appearance, and neither of them is like an $F_1$ bird. Partial sterility has already been reported in this cross by others (GHIGI 1908).

### "FREAK" MALLARD CROSSED WITH VARIOUS WILD SPECIES

In my 1915 paper I described a variety of domestic Mallard showing in the male an absence of chestnut breast and white neck-ring, and a black instead of a green wing speculum. The female was plain-colored about the head, and the young were jet black in down plumage. This variety behaved as a simple, clear-cut, Mendelian recessive, in crosses with Mallard stock, and showed no tendency to variation.

In 1915 it was thought of interest to cross these "freak" Mallards with other pure wild species, to see how this new Mallard variety would be affected by meeting another set of germ characters. Accordingly female "freaks" were mated both with pure wild Black Ducks and pure Australian Ducks with the following result:

#### *"Freak" Mallard × Black Duck*

The first cross (cross S, 1915) produced 5 males and one female, all normal and just like the birds of the Mallard × Black cross described in the former paper.

The next year the second, generation ($F_2$, cross L, 1916), produced five normal and six freak young, the latter black in down. When these grew up there remained:

| | FEMALES | MALES |
|---|---|---|
| Normal | 3 | 1 |
| Freak | 0 | 2 |

In addition one doubtful male (No. 2422) was obtained, looking like an $F_1$, but with a very dull-colored speculum. It was marked "normal" when in down plumage.

This series agrees with Mendelian expectation as far as it goes. The male "freaks" tend to segregate into two types, extreme Mallard with solid-green head and vermiculated breast, and a more female-like type, plain-colored all over. The doubtful male cannot be classified with certainty, but was probably a normal, slightly influenced by "freak" characters.

### *"Freak" Mallard × Australian Duck*

In 1914 a female "Freak" Mallard × male Australian (cross B, 1914) produced 4 males and 7 females, all normal $F_1$ types.

In 1915 and 1916 sister × brother matings produced 28 ducklings, 19 normals and 9 "freaks." On maturity, 3 "freak" males and 4 normal males were saved as specimens. Again no "freak" females reached maturity. There were as adults:

| | FEMALES | MALES |
|---|---|---|
| Normal | 6 | 11 |
| Freak | 0 | 4 |

The "freaks" are all clearly separable, both in downy plumage and as adults. The only thing which here runs counter to expectation is the great variation in "freak" males. They tend to segregate into a very Mallard-like "freak" type with solid-green head and vermiculated breast and abdomen, and another type looking like a female "freak" Mallard. The normal $F_2$ males show more segregation than that seen in the Mallard × Australian cross (PHILLIPS 1915), but none shows the solid-green Mallard-like heads of the "freak."

As far as these two crosses go they show that the "freak character," or set of inhibitors, whatever one chooses to call it, acts normally when

brought into contact with the germ-plasm of two other true species. It even appears to have the effect of increasing the segregation in $F_2$ hybrids. There may be a lethal factor which inhibits the development of $F_2$ "freak" females, or else kills them in the shell. One very interesting young bird ("Freak" Mallard × Australian $F_2$, No. 1991) which was lost before reaching adult plumage, showed a curious bilateral difference in first plumage. A note on August 1st, 1915, describes this bird as having the whole right side lighter colored than the left side, the difference showing especially on the wing and the occipital and orbital head-streaks. Possibly this bird may have been a mixed "freak" and normal type, or a gynandromorph.

The lack of virility of the "freak" stock was mentioned in 1915 and it was always very difficult to maintain the strain with pure "freak" (recessive) matings.

## DISCUSSION AND CONCLUSIONS

Looking over the various matings between species described here, and those described in former papers, let us see whether some order can be brought out of the apparent chaos. It was suggested in 1915 (p. 110) that in closely related forms there is, under experimental conditions, a nearer approach to orthodox Mendelism than in crosses between more widely separated species; and the present work has, I think, gone far enough to fully bear out this point.

I will arrange the various species matings made at Wenham in a column, beginning with the most distantly related generic crosses and ending with species most closely related.

Reeves × Prince of Wales Pheasant (PHILLIPS 1916)

Reeves × Ring-neck Pheasant (PHILLIPS 1913)

Intergeneric crosses between genera only distantly related.
Hybrids sterile. Reciprocal hybrids unlike.
Malformation or absence of sex organs in females.
The cross difficult to obtain on account of sterility of most of the eggs of parents.

Muscovy Duck × Mallard

Intergeneric cross said to produce unlike sterile reciprocal hybrids, but females sometimes lay eggs. (These reciprocal crosses were not produced by me. POLL 1910).

Silver Pheasant × Swinhoe (SMITH and HAIG-THOMAS 1913, and present paper)

A species cross accompanied by partial sterility of hybrids (found sterile by GHIGI 1908), and producing probably abnormal types of hybrids, but not a large enough series to draw definite conclusions.

Mallard Duck × Pintail (PHILLIPS 1915)

Intergeneric mating showing no sterility. The genera Dafila and Anas are closely related, and give results very different from the above-mentioned crosses. The parental colors and patterns differ, plumage character

complexes are unrelated, and $F_2$ hybrids show the smallest amount of segregation. Back-crosses show even less tendency to segregate than does the second generation. This is the best example of hybrids "breeding true."

Golden Pheasant × Lady Amherst (present paper)

A species cross between parents having several well marked alternative characters. Pattern in general the same, but colors very different. Hybrids show a moderate amount of segregation. On the theory of two closely related species, this mating ought to show a closer approach to simple Mendelian behavior than it does. This is possibly accounted for by the very complicated nature of the colors and color patterns in the parents. Back-crosses appear to show more segregation than $F_2$.

Black Kalij × Silver Pheasant (present paper)

A species cross where the parents have well marked alternative characters. Hybrids show no apparent sterility and $F_2$ exhibited a clear-cut type of segregation (see figure 5).

Mallard × Australian Duck (PHILLIPS 1915, and present paper)

A cross between two closely related species showing a moderate amount of segregation. Less segregation in back-crosses.

Mallard × Black Duck (PHILLIPS 1915, and present paper)

Comparable with last cross, but parent species more closely related and segregation somewhat pronounced.

Ring-neck × Prince of Wales Pheasant (PHILLIPS 1915)

A cross between two nearly related species, one of which, the Ring-neck, is probably not pure, but made up of *Phasianus colchicus* and *P. torquatus*. Alternate characters showing well marked segregation.

Mallard × Florida Duck (present paper)

Showing most extreme example of segregation thus far discovered in species crosses between either ducks or pheasants. Parental types are almost recovered from among 38 $F_2$ males. Back-crosses to Mallard parent produce a more uniform generation of nearly pure Mallard types. The Florida Duck must be supposed to represent, from the genetic point of view, substantially a Mallard, with the male secondary sex characters not expressed. It must thus be regarded, according to the breeding test, as more closely related to the Mallard than is the Black Duck, or the Australian Duck. (Figures 1 and 2.)

These differing results do not, of course, necessitate the consideration of a different mechanism of heredity, as the types used become more and more closely related in a genetic sense. The results of hybridization are in *appearance* different because of partial sterility in the most extreme matings, because of great numbers of factorial differences affecting the characters—but without sterility—in the next cases, and because of few factorial differences in the last case.

Two types of domestic variations in ducks have also been investigated. In one of these, the "East India" melanistic Mallards were shown to be dominant over pure Mallard stock (PHILLIPS 1915), but extracted normal Mallards entirely free from melanism have not been obtained from the cross. The ratio between extracted normals and "East Indias" remained,

however, a simple Mendelian one. The other variation investigated was the "freak" Mallard, a type acting in crosses as a pure Mendelian recessive unit character. The "freak" character, though behaving as a unit, has produced many profound changes in plumage, in down-coloring of young, and in viability and fertility. When crossed with other distinct species, the "freak" Mallards do not lose their identity, but actually appear to segregate more readily than normal Mallards in the same crosses.

We have, therefore, a gradation of results in species crosses starting with sterile monstrosities, whose sex organs may even be absent, and ending with species combinations showing, in spite of small numbers, such a near approach to parental types in the segregates, that their plumage characters may be supposed to differ from each other by only three or four principal factors.

How then shall we explain the behavior of stable hybrids like those between the Pintail and Mallard Ducks? My own preference would be the assumption of two character-complexes, very distantly related, and presenting such an enormous series of possibilities in $F_2$, that with the small numbers at hand only the very centre of the curve of frequency has thus far been brought to light in experiments. There is absolutely no evidence of infertility in this cross and therefore I see no reason to assume the presence of selective elimination due to balanced lethal factors.

In these species-crosses we have found no true sex-linked inheritance. In species crosses the more important plumage characters are not recombined independently, but nearly always reappear together, so that the most extreme individual in a series will contain several plumage characters of an extreme parental type. This does not always hold, however; and quite important characters at times become disassociated in hybrids, and crop up at random. This is seen in the matings between *Phasianus torquatus* and *P. principalis* and also in back-cross Gold-Amherst matings. As a rule, however, we may say that plumage characters of species, even for widely separated feather tracts, are closely linked.

The fact that complicated male color patterns *can* be at times broken up in inheritance, even in a very limited series of individuals, tends to show that some of the elements of the secondary sex color-patterns may be carried in separate chromosomes.

The series of crosses here reported give results in accordance with the views of those who believe that genetic factors are carried by the chromosomes. In the more distantly related species, the plumage patterns and colors are affected by so many factors that the majority of segregates are more or less intermediate; but in the more closely related species, decidedly

different patterns and colors may result because of the interaction of relatively few inherited factors. In the latter case the visual result of segregation will be more marked.

The independence of color factors, and factors underlying fertility and sterility, is striking, especially in ducks. Ducks afford interesting material because of the presence of fertility in wide crosses.

The sterile and unlike reciprocal hybrids between Reeve's and Ringneck Pheasants should be carefully investigated by a competent cytologist. The writer some years ago possessed a large amount of this material which was, unfortunately lost in the laboratory to which it was sent before it could be studied.

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# DICE-CASTING AND PEDIGREE SELECTION. EXPERIMENTS WHICH PICTURE MATHEMATICALLY CLOSE ANALOGIES BETWEEN DICE-CASTING AND CERTAIN BREEDING PHENOMENA

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## INTRODUCTION

There is a striking analogy between a situation which may be described on the several faces of a cube, and the actual potentiality of a given genotype to throw a number of phenotypes. By casting the dice according to given rules, the analogy may be carried further to the extent of illustrating the mathematical nature of filial regression, of rating pure lines, and of analyzing the nature of selection within them. In the improvement of domestic plants and animals, practical breeders, from time immemorial, have followed the empirical method of selection based upon somatic qualities. With the advent of modern genetics breeders are paying more attention to pedigree systems of improvement, and have often sought the aid of genetic analysis. The contrast between the outcome of selection based upon somatic qualities and of selection based upon attention to germinal constitution, may be shown clearly by dice-casting.

The several experiments which are here selected for description, including the recording, tabulation, and the analysis of data, may be completed in the course of a few hours. The only apparatus needed (figure 1) consists

of one dice cup and six blank dice, which latter may be labelled with pen or pencil to suit the particular experiment.

Figure 2 gives the surface patterns and lettering of the dice actually used in these demonstrations. The face values begin with 1 and continue up to 6 for die a; for die b they begin at 2 and continue up to 7, and so on through the series, die f beginning with 6 and ending with 11, the highest value.

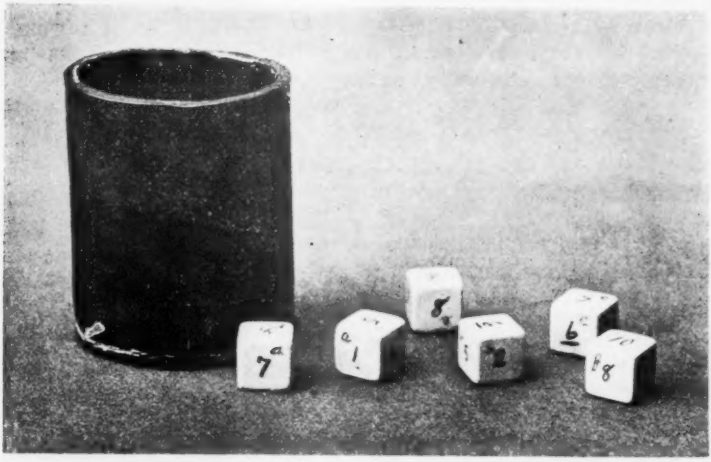

FIGURE 1.—Apparatus needed. One dice cup, and six blank dice which may be labeled with pen or pencil.

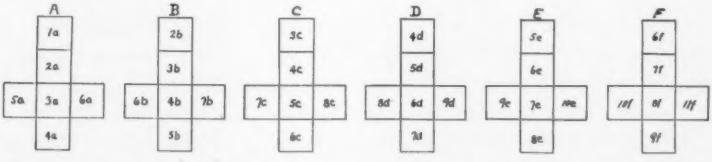


FIGURE 2.—Surface patterns and lettering of dice used in the experiments here described.

## BIOLOGICAL ANALOGIES

Table 1 presents a short statistical treatment of the possible somatic values, that is, the phenotypes, of the offspring of a given definite genotype. Each die of the whole series, a, b, c, d, e, and f, regardless of the values of its several faces, represents the definite germinal constitution, that is, the genotype, of a self-fertilized parent. Upon examination of the values

of the several faces of each die, the experimenter learns the potential range of value of each fraternity in the $f_1$ generation, derived from the several particular genotypes. If the demonstrator cares for a more elaborate system, he can use many-sided symmetrically or even irregularly shaped and weighted solids for the purpose of approaching, perhaps more nearly, the real biological situation. But in so doing, the directness and simplicity of the experiment would be somewhat sacrificed. It would probably not be worth making the change to the irregular or more complicated system, because the purpose of this demonstration is not to prove any biological fact, but, by analogy, to picture certain mathematical phenomena of biological facts and reactions already known.

TABLE 1

*Description of the six dice used, and their biological analogies.*

| The Die (ie. the genotype) Label all faces of same die with same letter. | The Die's Six Faces (ie. the several—in this case 6—possible somatic variation-values—phenotypes—of the offspring of a given definite germinal constitution—genotype—of a self-fertilized parent.) | | | | | | | | | | | Sum of face values of same die | Mean face value of same die |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| a | 1 | 2 | 3 | 4 | 5 | 6 | | | | | | 21 | 3.5 |
| b | | 2 | 3 | 4 | 5 | 6 | 7 | | | | | 27 | 4.5 |
| c | | | 3 | 4 | 5 | 6 | 7 | 8 | | | | 33 | 5.5 |
| d | | | | 4 | 5 | 6 | 7 | 8 | 9 | | | 39 | 6.5 |
| e | | | | | 5 | 6 | 7 | 8 | 9 | 10 | | 45 | 7.5 |
| f | | | | | | 6 | 7 | 8 | 9 | 10 | 11 | 51 | 8.5 |
| Face Value | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | For all 6 dice | |
| Frequency | 1 | 2 | 3 | 4 | 5 | 6 | 5 | 4 | 3 | 2 | 1 | @ faces = 36 | |
| F x V | 1 | 4 | 9 | 16 | 25 | 36 | 35 | 32 | 27 | 20 | 11 | @ face values = 216 | |
| | | | | | | | | | | | | Mean face value = 6 | |

← Somatic selectionists generally view in this direction. — Face values — ↑

← Pedigree selectionists generally, and pure line selectionists especially, view in this direction. →

← Potential values. →

By examining the several phenotypes (faces of the several dice), the experimenter may record their observed values, as was done in the preparation of table 1, which shows the distribution of face values among the several dice. He may then proceed to determine the sum of the several face values of the same die and the mean of such values. He may determine also the frequency of each face value as found in the whole collection of dice, and may proceed to calculate the mean value for the entire "potential population," that is for all of the face values of all of the dice.

It is not a complex picture of all that takes place in nature, but the barest and most direct analogy to the statistical essentials of the case, that we now desire. Analogies are useful for illustrating principles, but they all have their limitations in respect both to possible parallelism and to

usefulness. If all parallels were exact to the end, then, of course, the analogy would cease, and instead of having dice with numbers, we should have to deal with the genotype itself as found in actual living organisms. One advantage of dice as analogies is that an experimenter may pursue a number of "generations" in a few minutes, and may at the same time have "a peep behind the scenes," as it were, when he picks up the die, and examines and lists the whole range of "phenotypic" possibilities of the "offspring." The actual breeder at the beginning of his work does not know the nature of the genotype, that is to say, he at first, can see only the somatic aspect of the selected parent. With dice, however, the "genotype," or the potentiality for throwing a number of different values, can be determined by picking up the particular die and examining it. This insight is permissible because it makes the analysis of statistical results much clearer. A selected face value represents a parent, and the face values which come up from successive castings are possible $f_1$ offspring. Further one has the advantage of casting as many times as he chooses. In nature the greater the number of offspring, the earlier and more surely will the experimenter exhaust the phenotypic possibilities. Also, conversely, the smaller the number of such possibilities, the more quickly will they be found.

## PARENTS AND OFFSPRING. REGRESSION

Table 2 presents the next step in logical analysis. In this table there is given a mathematical picture of a "breeding experiment." The experimenter examines all of the "potential parents," and first classifies them according to "face" values. In column 2 he records the number of "parents" within whose "genotypes" each of the several different values are found. In the next column he records the names of all the "genotypes" which carry the various selected values. He then, by examination or casting, finds the mean value of all of the "potential offspring" of each particular "genotype." The results are recorded in column 4. He then casts each particular group of "genotypes" a hundred times. In column 5 he records the number of "offspring" resulting from such castings; in column 6 is recorded the mean actual value of all of the "offspring" registered in column 5. A comparison between the values in columns 4 and 6 shows the difference between the mean absolute "genotypic" values and the values actually attained in the particular experiment.

Practically, table 2 was constructed as follows: First the dice were laid on the table and carefully turned until all faces (in this case

on one die only) with the face value of 1, were found. Then these were turned up for "parents" and cast 100 times; the mean face value was found to be 3.58. Next the six dice were again laid on the table and all of them with 2's were sorted out. The latter faces were then turned up for

TABLE 2

*Data recording numbers and values of "parents" and their "offspring."*

| 1* Selected Face Values (i.e., Parents) | 2 Number of dice on which selected values are found. | 3 Name of dice. (i.e., Genotypes) on which selected values are found. | 4 Absolute mean value of faces, (i.e., of possible phenotypes.) | 5 Number of faces cast up by 100 castings of group (i.e., number of offspring.) | 6** In an actual experiment mean cast up face value (i.e., mean value of offspring.) |
|---|---|---|---|---|---|
| All 1's | 1 | a | 3.5 | 100 | 3.58 |
| All 2's | 2 | ab | 4.0 | 200 | 3.75 |
| All 3's | 3 | abc | 4.5 | 300 | 4.64 |
| All 4's | 4 | abc<br>a | 5.0 | 400 | 5.10 |
| All 5's | 5 | abc<br>de | 5.5 | 500 | 5.67 |
| All 6's | 6 | abc<br>def | 6.0 | 600 | 5.99 |
| All 7's | 5 | bc<br>def | 6.5 | 500 | 6.54 |
| All 8's | 4 | c<br>def | 7.0 | 400 | 7.04 |
| All 9's | 3 | def | 7.5 | 300 | 7.52 |
| All 10's | 2 | ef | 8.0 | 200 | 7.84 |
| All 11's | 1 | f | 8.5 | 100 | 8.37 |

"parents" and cast 100 times; the mean of their face values was found to be 3.75; and so on, thus selecting and casting through the series of 3's and 4's to the 11's.

Figure 3 uses the facts recorded in table 2 in such a manner as to demonstrate what is meant by such terms as "filial regression," "rever-

sion," "the drag of the race," and "the pull of mediocrity." The phenomena of dice-casting and of biological reproduction are treated statistically in the same manner. All of the data of table 2 were transferred to the regression plot. When the lines are duly connected up, we find

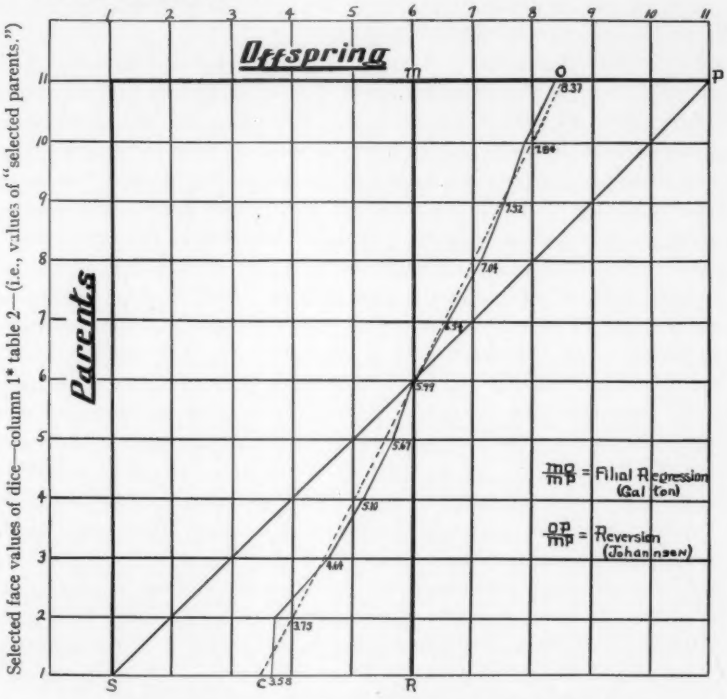


FIGURE 3.—Regression diagram, plotted from the data of table 2.

mR = mean of all face values (absolute) i.e., "mean of the race." (36 dice-faces.)
SP = line connecting values of selected face-value groups (i.e., "parental values.")
CO = regression line. (Dotted line theoretical, solid line actual, values found in 100 tossings of each group.)

a typical "filial regression" diagram. Now, by referring to table 1, the description of the dice used, we find that these regression values instead of running 3.58, 3.75, 4.64, 5.10, 5.67, 5.99, 6.54, 7.04, 7.52, 7.84, 8.37, actually run 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, making the theoretical regression line absolutely straight, and giving a "filial regres-

sion" of the value of 0.5. An indefinitely great number of tosses would of course, tend to convert the experimental values into the real values.

At this point the experimenter may, if he be interested, make a short clarifying excursion into the meaning of probable error. Here he knows the real values and by calculating the probable error for several series of experimental approximations which are determined from various numbers of samples, he sees with great clarity the relation between the number of samples and approximation to the truth.

The experimenter learns that with dice as well as with living organisms, the "offspring" of "genotypes" selected on the basis of extreme values will less nearly approximate their "parental" values and will more nearly approximate their "racial mean" than will the "offspring" of "genotypes" whose "somatic" values are found to lie nearer the mean value of the "race." By first casting the dice and preparing table 2, and then, in the regression diagram, plotting the data thus found, the experimenter receives a clear conception of what is meant by the term "filial regression," and he sees clearly several of the mathematical properties and the reasons for the existence of several associated phenomena. Thus, the regression line plotted from the experiment is straighter near the middle than at the extremities. This is due to the fact that while the number of tosses was the same for each "parental" value, the number of "parents" used varied from one on the extremes to six in the center, thus a regression value of the center group is based upon six times as many individual "offspring" as the regression values of the "parental" values 1 and 11.

Besides serving other purposes, regression is a sort of measure of the phenotypic range of a given genotype. If the regression be found very small, it may mean a number of things, for example, great homozygosity of the parents in reference to their own somatic standards, or a great "anti-mean" influence of environment upon the children. If the regression be very great, it may stand for considerable heterozygosity of the parents in reference to their own somatic measurements, or homozygosity in reference to the racial mean, possibly augmented in the children by a "racial mean-tending" influence of environment. This is nicely illustrated in DAVENPORT'S (1917) work on human stature. Among the children of tall parents he found but little regression, indicating relatively great constancy of germinal properties. Among children of shorter parents, however, he found a greater regression, indicating more complex and less constant hereditary natures.

It is obvious to the observer that figure 3, and the data from which it is constructed, could be used to good purpose for demonstrating the statistical nature of correlation.

## RATING PURE LINES

If we turn now to table 3, "The determination and rating of pure lines," we may, by continuing the view behind the scenes (that is, the examination of the dice used for parents), come to an understanding of the theory of

TABLE 3

*The determination and rating of pure lines. Letters describe genotypes and, in $F_1$, locate members of same pure line.*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $P_1$ | Let actual chance casting represent $P_1$ Phenotypes. | Order → | 1 | 2 | 3 | 4 | 5 | 6 |
| | | An actual chance casting | 4a | 5c | 6b | 6e | 8f | 9d |
| $F_1$ | Cast each die 10 times to find the approximate mean value of its several (6) faces. (i.e., to find the approximate mean value of the several phenotypic aspects of each selected genotype.) | The 10 castings, (i.e., values of the 10 offspring.) | 5a | 4c | 3b | 6e | 6f | 4d |
| | | | 1a | 3c | 5b | 6e | 10f | 8d |
| | | | 6a | 5c | 3b | 10e | 10f | 7d |
| | | | 2a | 5c | 7b | 7e | 7f | 8d |
| | | | 2a | 6c | 5b | 10e | 11f | 9d |
| | | | 5a | 6c | 3b | 10e | 7f | 8d |
| | | | 4a | 7c | 5b | 9e | 11f | 5d |
| | | | 5a | 8c | 6b | 8e | 11f | 8d |
| | | | 4a | 4c | 3b | 9e | 9f | 9d |
| | | | 6a | 4c | 7b | 7e | 7f | 5d |
| Pure-Line Determination. | $\Sigma$ $F_1$ values | | 40 | 52 | 47 | 82 | 89 | 71 |
| | mean $F_1$ values (10 offspring) | | 4.0 | 5.2 | 4.7 | 8.2 | 8.9 | 7.1 |
| | Order of mean values of $F_1$ (Compare with $P_1$ order.) | | $1_a$ | $3_c$ | $2_b$ | $5_e$ | $6_f$ | $4_d$ |
| | Absolute mean value of 6 faces of die, (i.e. $F_1$ phenotypic range, or mean value of the several "Pure Lines.") | | $3.5_a$ | $5.5_c$ | $4.5_b$ | $7.5_e$ | $8.5_f$ | $6.5_d$ |
| | Order of mean of absolute face values (i.e., mean of $F_1$ Phenotypes.) (Compare with above order-ranks.) | | $1_a$ | $3_c$ | $2_b$ | $5_e$ | $6_f$ | $4_d$ |

the pure line, which appears to be a real biological principle of practical value to the breeder. Let us cast the six dice on the table. In the actual experiment recorded in table 3, they turned up as follows: 4a, 5c, 6b, 6e, 8f, and 9d. They are next arranged in order of values, regardless of the meaning of the letters. 4a was considered a $p_1$ parent, and cast 10 times for the 10 $f_1$ offspring. The mean value of these offspring was found to be 4.0. Similarly the remaining mean values ran: of 5c, 5.2; of 6b, 4.7; of 6c, 8.2; of 8f, 8.9; of 9d, 7.1. Then by examining the labels the mean values of the $p_1$ parents were found in the following ascending order; a, c, b, e, f, d. The mean value of the $f_1$ offspring of each parent gave the following order: a, b, c, d, e, f,—the actual ascending values which we found in earlier experiments. It is noticed that whereas the array of somatic or phenotypic values in the $p_1$ generation gave one order of values to the several letters, this order was a false one, so far as the potentialities of the line are concerned; the $f_1$ order is the true one.

We may say, then, that the self-fertilized "offspring" of face 4a constitute "pure line a," of face 5c "pure line c," and so on. Now the principal characteristic of the pure line is that, so far as hereditary constitution, that is, the genotype, is concerned, one individual member is the same as another, and consequently will produce the same sort of offspring. This is tested in tables 4 and 5.

## SELECTION WITHIN PURE LINES

The next logical step is registered in experiment 1, table 4. The experimenter selects a low "$p_1$" value, as face 1, die a. He casts the selected die twenty-five times in order to find the approximate mean value of the "$f_1$ offspring," and also to determine the range of possibility in variation among the offspring. He selects the lowest value of these twenty-five "offspring" as a "parent," and casts the die that bore it twenty-five times for the "$f_2$ generation," and continues the same process and records through the "$f_3$ generation," (or longer if he be skeptical). He finds that the mean of the third "generation" is 3.2, that the mean of the whole line is 3.41. Compared with this he selects face value 6 from the die of "pure line a," and selects for high values. He finds the mean value of the third "filial generation" to be 4.28, and of the whole line to be 3.88. While he began on the one hand, with 1, and the other with 6, he has at the end of 3 generations mean values of 3.41 and 3.88 respectively. In real life, a selectionist might believe that he was making some progress, but our knowledge of the dice in this particular analogy shows us that difference in the direction of selection is a mere chance. This slight

temporary difference in favor of the direction of selection is one of the characteristic phenomena of pure-line manipulation. If we should continue it through many generations, the "line 1a" would many times fluctuate

TABLE 4

*The nature of selection within the pure line. Each figure represents one actual casting of the die, which in turn represents one offspring.*

| Actual Experiment No.1. Showing the Futility of Selection within the Pure Line. | | | Actual Experiment No. 2. Showing that A Small Value from a Superior Line Constitutes a Better Parent than an even Higher Value from an Inferior Line. | | |
|---|---|---|---|---|---|
| Selected $P_1$ | Face (i.e., Somatic) value = 1 Member of Pure Line a (Select for low values) | Face (i.e. Somatic) value = 6 Member of Pure Line a (Select for high values) | Selected $P_1$ | Face (i.e., Somatic) value = 4 Member of Pure Line d (Select for high values) | Face (i.e., Somatic) value = 7 Member of Pure Line b (Select for high values) |
| $F_1$ | 4 2 4 *1 3<br>4 2 3 1 4<br>5 3 4 5 6<br>3 2 5 1 4<br>6 1 1 6 5<br>25 Casting values i.e. "Offspring" Range = 1 to 6 Mean = 3.4 | 5 5 4 1 5<br>2 5 1 4 3<br>2 6 5 1 1<br>6 2 6 *6 5<br>1 2 6 3 5<br>25 Casting values i.e. "Offspring" Range = 1 to 6 Mean = 3.68 | $F_1$ | 7 4 6 6 8<br>6 7 5 4 *9<br>6 8 5 8 8<br>6 5 7 9 4<br>5 9 7 6 7<br>25 Casting values i.e. "Offspring" Range = 1 to 9 Mean = 6.48 | 3 5 5 4 5<br>2 5 3 7 6<br>5 2 3 6 7<br>4 4 3 3 6<br>3 4 *7 6 5<br>25 Casting values. i.e. "Offspring" Range = 1 to 7 Mean = 4.6 |
| $F_2$ | 4 *1 2 4 5<br>4 5 3 6 2<br>3 5 3 1 5<br>2 3 4 6 1<br>6 4 5 5 2<br>25 Casting values i.e. "Offspring" Range = 1 to 6 Mean = 3.64 | 4 5 3 1 *6<br>3 6 1 1 6<br>3 3 1 2 5<br>3 5 5 6 3<br>1 5 5 5 4<br>25 Casting values i.e. "Offspring" Range = 1 to 6 Mean = 3.68 | $F_2$ | 7 7 4 7 *9<br>6 6 7 5 6<br>7 4 8 7 8<br>9 4 4 7 7<br>6 5 6 7 8<br>25 Casting values i.e. "Offspring" Range = 1 to 9 Mean = 6.44 | 7 5 4 5 *7<br>3 6 4 5 6<br>4 5 5 4 3<br>2 4 4 5 7<br>3 3 2 4 5<br>25 Casting values i.e. "Offspring" Range = 1 to 7 Mean = 4.6 |
| $F_3$ | *1 4 1 2 6<br>5 2 4 5 3<br>3 5 1 1 1<br>4 3 2 3 5<br>4 3 5 5 2<br>25 Casting values i.e. "Offspring" Range = 1 to 6 Mean = 3.2 | 6 6 6 2 2<br>6 6 4 5 6<br>4 4 6 5 3<br>5 5 1 1 5<br>4 5 *6 2 2<br>25 Casting values i.e. "Offspring" Range = 1 to 6 Mean = 4.28 | $F_3$ | 7 8 9 6 5<br>5 6 6 7 4<br>5 6 7 6 6<br>7 9 9 *9 8<br>7 6 6 7 7<br>25 Casting values i.e. "Offspring" Range = 1 to 9 Mean = 6.72 | 3 3 3 4 4<br>7 6 5 4 4<br>5 4 3 5 2<br>2 6 4 6 7<br>3 5 *7 3 3<br>25 Casting values i.e. "Offspring" Range = 1 to 9 Mean = 4.32 |
| | Range of Line 1 to 6 Mean of Line 3.41 | Range of Line 1 to 6 Mean of Line 3.88 | | Range of Line 1 to 9 Mean of Line 6.54 | Range of Line 1 to 7 Mean of Line 3.50 |

NOTE:—* indicates parents selected for next generation.

in mean value, past "line 6a," but of course, the two real mean values are infallibly fixed at identity. In the case of dice-casting a little reflection shows the reason for this apparent progress. Possibly analogous causes exist in other selection phenomena within living species.

Now let the experimenter cast the six dice representing six $p_1$ individuals (experiment 2, table 4). If he finds "die d" showing a face value of 4, and die b a face value of 7 (as actually came up for the demonstration here recorded), he knows that he has a situation which he desires, because "line b" is of lower average value than "line d." He then repeats the experiment just described in selecting for high values through three or more generations. At the end of the experiment he finds that, regardless of the values of the original "parents," the true mean value of the line is soon apparent. Indeed here again the principal story is told in the "$f_1$ generation," and indeed the "$f_1$ generation" would tell it completely if there were enough castings made. Carrying the selection through further "generations" only serves to verify and emphasize this phenomenon. In the inferior "line b" the selection started with the chance high value 7, and ended with the mean value 4.32, approximating the true mean 4.5 of the line. In the "better line d," a chance low value 4 ended in a mean valueof 6.54, true mean 6.5. Thus the principal practical corollaries from selection read as follows:

a. A small value from a superior line constitutes a better parent than an even higher value from an inferior line.

b. Selection within a pure line is futile. In both cases selection only uncovers the mean potential value of the line; it does not create. We must cross-breed, that is, introduce new blood, or hope for mutations, if we would change or improve it further.

If the experimenter cares to make the pure-line demonstration a little more vivid historically, he may parallel JOHANNSEN'S famous "19-bean experiment," by labelling nineteen dice as follows: 1, 2, 3, 4, 5, 6; 2, 3, 4, 5, 6, 7; and so on to 19, 20, 21, 22, 23, 24. The processes described in tables 3, 4, and 5, may then be followed out.

## SELECTION: SOMATIC BASIS

Proceed now to table 5, "Selection based upon somatic qualities." This table was built upon four actual dice-casting experiments. In each case the selection was begun with six "unknown" dice ("self-fertilized parents"), and was then continued, seeking to establish (a) a strain of lowest average value, and (b) one of highest average value. To begin each experiment, first the six dice were cast; the upturned faces represented the six different parental values. The lowest of these "parental values," face 3 of die a, was taken for the "ancestry" of the "$f_1$ generation" in seeking to establish a low "strain;" for the high "strain" face 7 of die e was selected. Ten castings of each of the selected "$p_1$ parents" gave

the ten "$f_1$ offspring." Then in the low "strain" the lowest of these ten "$f_1$ offspring" was selected for the "parent of the $f_2$ generation;" and the highest of the "offspring" of the high "strain" was selected for the "parent" of the "$f_2$ high strain " This type of casting and selection was continued through four generations.

The comments which are written in the last column under each experiment are those which the practical breeder would be apt to make in seeking to interpret what he had actually found. We must remember that without pedigree analysis, the actual breeder sees only somatic values; the genotypic constitutions (represented by letters and figures on the dice) which we have added to guide us through these experiments, are unknown to him.

The dice-caster sees at first-hand that his selection had only sorted out and brought to light the highest values at first hidden, and that it had not created anything that did not previously exist. He learns also that a single experiment of this type does not suffice to reach the limits of possibilities of selection; that the weakness in this system consists principally in its dependence upon chance in the "$p_1$ generation" to isolate the highest and lowest "genotypic" values. Thus in the four experiments here tabulated, in seeking the lowest "strain," genotype a, which in fact constituted the lowest "strain," was segregated three times, and the next lowest, "genotype b," once. In seeking the highest strain, genotype f, the actual highest was isolated twice, genotype e, the next highest, once, and genotype d, the next highest after e, once. In this system there is, then, a relation between the number of potential strains and the number of experiments necessary for their determination.

It would seem that the practical breeder, by following his usual somatic methods of selection, works largely in the dark. He, nevertheless, as the splendid results in plant and animal breeding show, has made substantial progress. A closer attention to genotypic analysis would doubtless result in more rapid progress, as we have demonstrated in tables 3 and 4.

The tables and diagrams here presented consider principally the case of pure lines, that is, of the decendants of self-fertilized individuals. Originally JOHANNSEN (1903) defined a pure line as "the progeny of a homozygous, self-fertilized individual." Later JENNINGS modified, or rather extended the definition to mean "all of the descendants of a single self-fertilized individual." In leaving out the requisite "homozygous," the change was not so great as might at first be expected, because continued self-fertilization makes rapidly for homozygosity (see EAST and JONES 1919, chapter 5; JENNINGS 1916, 1917; PEARL 1917).

## CONSTRUCTION OF TABLE 5

*Selection based upon somatic qualities*

A. Purpose: In each experiment beginning with the same 6 unknown dice, (i.e., self-fertilized parents), and continuing selection, without knowing number of sides or their labels (i.e., the range or number of potential types of offspring, save as they appear in the tossing, i.e., breeding), seek to establish (a) a strain of *lowest* average value, and (b) one of *highest* average value.

B. Instructions:

1. In $p_1$ generation cast all dice and arrange in somatic (i.e., face) order.
2. Select as parents the individual soma, (i.e., face) with the lowest value for founding the (−) strain, and with the highest value for founding the (+) strain.
3. Continue this system. In each succeeding generation cast the selected parent 10 times to represent the 10 offspring.
4. The soma, (i.e., face) bearing most strongly in the desired direction (marked *) is saved for breeding, (i.e., for casting the next generation); the remainder are measured to find the mean of the generation, and are then discarded.

## TABLE 5

*Four actual dice-casting experiments illustrating the nature of selection based upon somatic qualities.*

### Actual Experiment No. 1

| | strain | | | | | strain | | |
|---|---|---|---|---|---|---|---|---|
| $P_1$ | *3a | 4c | 4d | 5b | 6f | *7e | mean of $P_1$ = 4.83 | |
| $F_1$ | *1a | * = Save for "breeding" Measure, then discard the remainder of each generation | | | | 12e | Notes | |
| | 2a | | | | | 7e | | |
| | 1a | | | | | 10e | | |
| | 4a | | | | | 7e | | |
| | 3a | | | | | 9e | | |
| | 2a | | | | | 8e | | |
| | 2a | | | | | 8e | | |
| | 2a | | | | | 7e | | |
| | 3a | | | | | 5e | | |
| | 2a | | | | | mean = 7.9 | | |
| | mean = 2.2 | | | | | | Real progress. | |
| $F_2$ | 6a | | | | | 6e | | |
| | 6a | | | | | 7e | | |
| | 1a | | | | | 7e | | |
| | 5a | | | | | 8e | | |
| | *1a | | | | | 7e | | |
| | 2a | | | | | 6e | | |
| | 6a | | | | | 5e | | |
| | 4a | | | | | 8e | | |
| | 2a | | | | | 5e | | |
| | 1a | | | | | *10e | | |
| | mean = 3.4 | | | | | mean = 6.9 | A little discouraging 7.5 maybe a bad season." | |
| $F_3$ | 3a | | | | | 8e | | |
| | 3a | | | | | 6e | | |
| | 2a | | | | | 7e | | |
| | *1a | | | | | *10e | | |
| | 1a | | | | | 5e | | |
| | 5a | | | | | 7e | | |
| | 3a | | | | | 5e | | |
| | 6a | | | | | 8e | | |
| | 6a | | | | | 7e | | |
| | 6a | | | | | 5e | | |
| | mean = 3.6 | | | | | mean = 6.8 | No sign of progress. Wait and see." | |
| $F_4$ | 4a | | | | | 8e | | |
| | *1a | | | | | 7e | | |
| | 5a | | | | | 8e | | |
| | 3a | | | | | 8e | | |
| | 5a | | | | | 6e | | |
| | 5a | | | | | 8e | | |
| | 3a | | | | | 5e | | |
| | 5a | | | | | *10e | | |
| | 3a | | | | | 8e | | |
| | 5a | | | | | 6e | | |
| | mean = 3.9 | | | | | mean = 7.3 | Some improvement in + strain, but continued debacle in − strain. But it is apparent that all of the progress was made in the $F_2$ generation. | |
| | mean of Line 3.38 | | | | | mean of Line 7.22 | | |

### Actual Experiment No. 2

| | strain | | | | | | | strain | | |
|---|---|---|---|---|---|---|---|---|---|---|
| $P_1$ | *2a | 4c | 4f | 7b | 7e | *8d | mean of $P_1$ = 2.66 | | | |
| $F_1$ | 3a | | | | | 5d | Notes | | | |
| | 5a | | | | | 6d | | | | |
| | 6a | | | | | 5d | | | | |
| | 2a | | | | | 9d | | | | |
| | 6a | | | | | 9d | | | | |
| | 2a | | + Strain Parent of $F_2$ | | | 8d | | | | |
| | 2a | | | | | *9d | | | | |
| | 4a | | | | | 8d | | | | |
| | 1a | | − Strain Parent of $F_2$ | | | 4d | | | | |
| | *1a | | | | | 9d | | | | |
| | mean = 3.2 | | | | | mean = 7.2 | Real progress but not so marked as in Experiment No. 1 | | | |
| $F_2$ | 2a | | | | | 4d | | | | |
| | 2a | | | | | 9d | | | | |
| | 4a | | | | | 5d | | | | |
| | 1a | | | | | 9d | | | | |
| | 4a | | | | | 7d | | | | |
| | 3a | | | | | 4d | | | | |
| | 4a | | | | | 9d | | | | |
| | *1a | | | | | 5d | | | | |
| | 5a | | | | | *9d | | | | |
| | 6a | | | | | 4d | | | | |
| | mean = 3.2 | | | | | mean = 6.5 | − strain Stationary. + strain degenerates a little. | | | |
| $F_3$ | 6a | | | | | 5d | | | | |
| | 2a | | | | | 5d | | | | |
| | 6a | | | | | 7d | | | | |
| | *1a | | | | | 7d | | | | |
| | 3a | | | | | *9d | | | | |
| | 5a | | | | | 4d | | | | |
| | 6a | | | | | 7d | | | | |
| | 1a | | | | | 6d | | | | |
| | 3a | | | | | 8d | | | | |
| | 1a | | | | | 7d | | | | |
| | mean = 3.4 | | | | | mean = 6.5 | − strain loses some of its smallness. + strain constant. | | | |
| $F_4$ | *1a | | | | | 5d | | | | |
| | 3a | | | | | 4d | | | | |
| | 5a | | | | | 5d | | | | |
| | 3a | | | | | 6d | | | | |
| | 1a | | | | | 8d | | | | |
| | 4a | | | | | 5d | | | | |
| | 3a | | | | | 9d | | | | |
| | 4a | | | | | *9d | | | | |
| | 1a | | | | | 4d | | | | |
| | 6a | | | | | 8d | | | | |
| | mean = 3.1 | | | | | mean = 6.3 | Encouraging drop in value of − strain. Discouraging loss in + strain. | | | |
| | mean of Line 3.22 | | | | | mean of Line 6.32 | | | | |

### Actual Experiment No. 3

| | strain | | | | | | strain | | |
|---|---|---|---|---|---|---|---|---|---|
| $P_1$ | *1a | 2b | 4d | 4c | 9e | *9f | mean of $P_1$ = 4.83 | | |
| $F_1$ | 4a | | | | | 6f | Notes | | |
| | 5a | | | | | 7f | | | |
| | 4a | | | | | 7f | | | |
| | 1a | | | | | 10f | | | |
| | 3a | | | | | *11f | | | |
| | 6a | | | | | 7f | | | |
| | 3a | | | | | 11f | | | |
| | *1a | | | | | 8f | | | |
| | 2a | | | | | 9d | | | |
| | 3a | | | | | 6f | | | |
| | mean = 3.1 | | | | | mean = 8.0 | − strain about as in Experiment No. 2. +strain gain, it appears for the first time. Save it for seed." | | |
| $F_2$ | 3a | | | | | 11f | | | |
| | 1a | | | | | 6f | | | |
| | 2a | | | | | *11f | | | |
| | 5a | | | | | 10f | | | |
| | *1a | | | | | 8f | | | |
| | 6a | | | | | 6f | | | |
| | 5a | | | | | 10f | | | |
| | 5a | | | | | 8f | | | |
| | 2a | | | | | 7f | | | |
| | 5a | | | | | 8f | | | |
| | mean = 3.4 | | | | | mean = 8.5 | − strain loses some of its smallness. +strain improves. | | |
| $F_3$ | 4a | | | | | *11f | | | |
| | *1a | | | | | 7f | | | |
| | 6a | | | | | 6f | | | |
| | 6a | | | | | 9f | | | |
| | 4a | | | | | 7f | | | |
| | 4a | | | | | 6f | | | |
| | 5a | | | | | 10f | | | |
| | 4a | | | | | 10f | | | |
| | 6a | | | | | 6f | | | |
| | 1a | | | | | 9f | | | |
| | mean = 4.1 | | | | | mean = 8.1 | Continued gain in − strain. Loss in + strain, but still greater than anything in Experiments Nos. 1 or 2. | | |
| $F_4$ | 1a | | | | | 10f | | | |
| | 3a | | | | | 10f | | | |
| | 1a | | | | | 9f | | | |
| | 6a | | | | | 9f | | | |
| | 3a | | | | | 8f | | | |
| | 6a | | | | | *11f | | | |
| | 4a | | | | | 9f | | | |
| | *1a | | | | | 8f | | | |
| | 5a | | | | | 7f | | | |
| | 2a | | | | | 10f | | | |
| | mean = 3.2 | | | | | mean = 9.1 | Original $F_1$ value in − strain. Greatest value yet in + strain. The most successful generation. Possibly continued breeding along these lines will result in the sought for values. | | |
| | mean of Line 3.67 | | | | | mean of Line 8.33 | | | |

### Actual Experiment No. 4.

| | strain | | | | | strain | | |
|---|---|---|---|---|---|---|---|---|
| $P_1$ | 3b | 3c | 3a | 7d | 8e | 11f | mean of $P_1$ = 4.83 | |
| $F_1$ | 4b | | | | | 11f | Notes | |
| | 6b | | | | | 11f | | |
| | 7b | | | | | 11f | | |
| | 4b | | | | | 7f | | |
| | 4b | | | | | 11f | | |
| | 3b | | | | | 11f | | |
| | 5b | | | | | 9f | | |
| | 7b | | | | | 8f | | |
| | 4b | | | | | *11f | | |
| | *2b | | | | | 6f | | |
| | mean = 4.6 | | | | | mean = 9.6 | Poorest results with − strain, but best with + Evidently a thrifty season." | |
| $F_2$ | 3b | | | | | 11f | | |
| | 6b | | | | | 9f | | |
| | 6b | | | | | 7f | | |
| | 3b | | | | | 11f | | |
| | 6b | | | | | 11f | | |
| | 7b | | | | | *11f | | |
| | 7b | | | | | 8f | | |
| | 4b | | | | | 8f | | |
| | *3b | | | | | 11f | | |
| | 6b | | | | | 7f | | |
| | mean = 5.1 | | | | | mean = 9.1 | Both strains move in the undesired direction. | |
| $F_3$ | 6b | | | | | 11f | | |
| | 7b | | | | | 7f | | |
| | 7b | | | | | 11f | | |
| | 6b | | | | | 11f | | |
| | 3b | | | | | 11f | | |
| | 5b | | | | | 9f | | |
| | *2b | | | | | *11f | | |
| | *2b | | | | | 7f | | |
| | 6b | | | | | 11f | | |
| | 7b | | | | | 8f | | |
| | mean = 5.1 | | | | | mean = 9.4 | − strain constant. + strain shows a little recovery. | |
| $F_4$ | 7b | | | | | 9f | | |
| | 4b | | | | | 8f | | |
| | 3b | | | | | 7f | | |
| | 7b | | | | | 6f | | |
| | 5b | | | | | *11f | | |
| | *2b | | | | | 10f | | |
| | 7b | | | | | 10f | | |
| | 7b | | | | | 10f | | |
| | 4b | | | | | 11f | | |
| | 3b | | | | | 8f | | |
| | mean = 4.9 | | | | | mean = 9.0 | Both strains less desirable than in $F_3$. | |
| | mean of Line 4.92 | | | | | mean of Line 9.28 | | |

But these experiments with dice need not be confined to pure lines. While pure-line phenomena are especially easy to parallel in dice-casting, bisexual heredity, even in the widest crosses, may be experimentally paralleled by appropriate dice-casting. Within bisexual species, let a single die represent the range of the combined genotypes of the two parents. In some respects the two genotypes will overlap; this narrows the total range; in others they will not; this extends it. If a solid with more than six faces be used, the several faces should be labelled with figures representing the range of possible values of offspring of a given pair of parents. This range will naturally vary greatly with different matings, but in each case will be complicated by such factors as Mendelian heterozygosity, intra-zygotic chemical reaction, due to the contact in the fertilized zygote of normally non-compatible elements, lethal factors, mutations regardless of size, crossing over, non-disjunction, deficiency, contamination, and other mechanical chromosomal phenomena, difference in environment furnished to offspring, and difference in response to environment of differently organized offspring. The whole range of value in the offspring will, of course, be limited by the possibilities of the physiological complex which heredity and environment furnish them, but, regardless of the cause of the range of phenotype in the offspring, we have simply to record these limits on the faces of the dice and proceed with the selection process exactly as in the case of pure lines. It would indeed be a complicated process, and would probably lose in clarity what it would gain in parallelism. Nor is the present scheme of dice-casting especially well adapted to demonstrating the statistical proportion of Mendelian behavior; coin-tossing is the scheme *par excellence* for this.

## SUMMARY

The statistical analogy between dice-casting and certain breeding phenomena is in many respects very close, and may be pushed as far as one may care to devise appropriate parallel mathematical contrivances.

The demonstrations described in this paper are mathematical—not physiological—pictures of only a few of the many facts and processes with which genetics deals. Practically, the value of these experiments consists in presenting concretely a situation in which the experimenter may, by handling the dice, casting them at will and recording and analyzing the data thus found, go to the root of the causes of the statistical phenomena with which he is dealing. This familiarity is denied in statistical studies dealing with the complexities of actual life; for the statistical meaning of the latter we are confined to logical deductions based upon similarity of

behavior to processes like dice-casting which we can follow throughout with the eye.

There are, of course, many differences between dice-casting and breeding phenomena, but they have this in common, in both dice-casting and organic reproduction the experimenter is permitted to see, at first, only a single value (the phenotype in life; the upturned face if a die) in a given individual, but if he continues the processes (dice-casting or breeding) long enough, he can test the range of variability possible to the "offspring" of the original "parental" individual. All of the essential external phenomena of schemes of selection and the mathematical treatment of their counts and measures may be clearly shown with dice. The parallel is especially clear-cut if the analogy be to pure lines.

Tables 1 and 2 and figure 3 present, in systematic form, from dice-casting data, the schemes usually followed in recording measurements for studies in variation, regression, and correlation in living organisms. One experiment (table 3) tests the "breeding properties" of a given individual here represented by the several face values of a single die. The demonstration described in table 4 shows how the selective processes, without creation, secures the highest values potential within a particular line or strain. Table 4 demonstrates also that in mongrel stock there is no essential relation between a particular somatic value and the breeding qualities of its possessor, and that for breeding purposes a low value from a good strain is better than a high value from a poor strain. We thus see that selection, as its name implies, only sorts; it does not necessarily create anything, much less does it create in the direction of its own wish. Table 5 shows the nature of selection based upon somatic qualities as contrasted with selection based upon a knowledge of the germ-plasm.

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# STERILITY IN WHEAT HYBRIDS. I. STERILITY RELATIONSHIPS AND ENDOSPERM DEVELOPMENT[1]

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## INTRODUCTION

Partial or complete sterility is of frequent occurrence in species crosses. Within the same genus certain species are sterile when crossed while other combinations are completely fertile. Partial sterility may cause the elimination of certain classes of gametes and prevent desired recombinations of characters in the second generation. Sterility may also cause an abnormal vegetative development. It is therefore desirable to determine the sterility relationships of the species of economic genera.

The present study of the genus Triticum will include: (1) the endosperm development in fertile and sterile crosses; (2) sterility in species crosses as determined by grains set and pollen counts; (3) hybrid vigor of $F_1$ plants in relation to sterility; and (4) size and variability of pollen grains in parents and $F_1$ plants. The second generation of species crosses and the cytological basis for sterility in wheat crosses will be treated later.

The species of wheat are divided by SCHULZ (see VON TSCHERMAK 1914) into three groups as follows:

[1] Papers from the Biological Laboratory, MAINE AGRICULTURAL EXPERIMENT STATION, No. 137.

1. The Einkorn group, consisting of *Triticum aegilopoides* and *T. monococcum*.
2. The Emmer group, consisting of *T. dicoccoides*, *T. dicoccum*, *T. durum*, *T. turgidum* and *T. polonicum*.
3. The *vulgare* group, consisting of *T. spelta*, *T. compactum*, *T. vulgare* and *T. capitatum*.

This grouping is in accord with serological relationships as determined by ZADE (1914). ZADE noted that the serum of *T. polonicum* reacted more strongly with the antigen of *T. durum* than with *T. turgidum* and *T. dicoccum*. He also called attention to the fact that in general the members of the Emmer group are resistant to rust while the *vulgare* group is susceptible.

VAVILOV (1914) classified the species of wheat according to their susceptibility or resistance to disease. His classification, applied to conditions found in Russia, is as follows:

In relation to *Puccinia triticina* Eriks.

| *Susceptible* | *Resistant* | *Perfectly immune* |
|---|---|---|
| *T. vulgare* (a few immune races) | *T. durum* | |
| *T. compactum* | *T. polonicum* | *T. monococcum* |
| *T. spelta* | *T. turgidum* | |

*T. dicoccum* has both susceptible and immune races. Practically the same relationship is indicated in respect to susceptibility to *Erysiphe graminis*.

VON TSCHERMAK (1914) and others have determined the degree of fertility of various species crosses. Following are VON TSCHERMAK'S results with wheat crosses:

1a. Einkorn × *vulgare* group, fully sterile.
1b. Einkorn × Emmer group, almost fully sterile.
2a. *T. dicoccoides* and *T. dicoccum* × Spelt
2b. *T. dicoccoides* and *T. dicoccum* × *T. vulgare*, *T. compactum* and *T. capitatum* } all weakly fertile.
2c. *T. dicoccoides* and *T. dicoccum* × *T. durum*, *T. turgidum* and *T. polonicum*, weakly fertile.
2d. *T. dicoccum* × *T. dicoccoides*, weakly fertile.
2e. *T. polonicum* × *T. vulgare*, *T. compactum* and *T. capitatum*, not fully fertile.
3a. *T. durum*, *T. turgidum* and *T. polonicum* × *T. spelta*, weakly or fully fertile.
3b. *T. durum* and *T. turgidum* × *T. vulgare*, *T. compactum*, and *T. capitatum*, fully fertile.
3c. *T. spelta* × *T. vulgare*, *T. compactum*, and *T. capitatum*
3d. *T. durum*, *T. turgidum*, and *T. polonicum*, *inter se*
3e. *T. vulgare*, *T. compactum*, and *T. capitatum*, *inter se* } all fully fertile.

The Emmer group as used by VON TSCHERMAK includes *T. dicoccum*, *T. durum*, *T. turgidum*, and *T. polonicum*. The *vulgare* group includes *T*

*spelta*, *T. vulgare*, *T. compactum* and *T. capitatum*. *T. polonicum* is considered more closely related to *T. durum* than is *T. turgidum* or *T. dicoccum*.

BLARINGHEM (1914) crossed Einkorn with *T. durum* and *T. turgidum* and obtained $F_1$ plants. Some grains set on the $F_1$ plants, and in later generations some individuals were fertile. TSCHERMAK and others attribute the development of grains of the $F_1$ of Einkorn-wheat crosses as possibly due to wind-pollination.

FREEMAN (1919) studied the inheritance of quantitative characters in a cross between *T. durum* and *T. vulgare*. He found some sterility and notes the shriveled grains in the first and second generations. He also found that tall $F_2$ plants with wrinkled seed (like the $F_1$) gave a more variable $F_3$ in respect to height than tall $F_2$ plants with smooth seeds (like the parents). The plump $F_2$ grains also gave taller $F_3$ plants.

HAYES, PARKER and KURTZWEIL (1920) used crosses of *T. vulgare* with *T. durum* and *T. dicoccum* in breeding for rust resistance. They found sterility in the $F_1$ plants and show the amount of pollen abortion. In the cross between Emmer and *T. vulgare* the pollen sterility is less than in the $F_1$ of *T. vulgare* $\times$ *T. durum*. The same sterility relationship is indicated by the percentage of seed set on $F_1$ plants.

Crosses between wheat and rye are difficult to make and very few seeds set on the $F_1$ plants. LEIGHTY (1920) found a number of natural wheat-rye hybrids and in most cases they were completely sterile. Nineteen plants produced only 40 seeds or about 1 percent of the flowers set seed.

A number of other writers have described wheat-rye hybrids and in all cases few grains were found on the $F_1$ plants.

## MATERIALS AND METHODS

The wheat species and varieties used in this study were obtained from E. F. GAINES, of the WASHINGTON AGRICULTURAL EXPERIMENT STATION. The following species are represented:

*Triticum vulgare*

1. Pacific Coast Bluestem, a wheat of Australian origin.
2. Amby, a true beardless wheat recently introduced from Australia.
3. Marquis, a well known wheat introduced by the OTTAWA STATION of Canada.

*Triticum compactum*

Washington Hybrid 143, a segregate from a cross of White Track $\times$ Little Club, made by Dr. SPILLMAN in 1898.

*Triticum durum*

Kubanka, a Russian wheat introduced by M. A. CARLETON of the U. S. DEPARTMENT OF AGRICULTURE.

*Triticum turgidum compositum*
Alaska, a bearded wheat with branching head.
*Triticum polonicum*
Polish, a bearded wheat with very long glumes and grain.
*Triticum monococcum.*
Einkorn.

Pure lines of Einkorn (*T. monococcum*) and Alaska as well as the $F_1$ hybrids were obtained from Dr. ZINN of the MAINE AGRICULTURAL EXPERIMENT STATION.

The varieties of wheat used in this work represent six species. These six species can be divided into three groups. First, *Triticum vulgare* and *Triticum compactum* which are fertile *inter se;* and second, *Triticum durum*, *Triticum turgidum* and *Triticum polonicum* which are also cross-fertile *inter se*. Members of the first group crossed with members of the second group result in partially sterile hybrids. For convenience the first group will be classed as the *vulgare* group, the second group as the Emmer group. Einkorn (*T. monococcum*) constitutes a third sterility group, but this species was not used at the beginning of the present study.

The crosses for this study were made in Illinois in 1919. Wheat scab was unusually prevalent and all grain obviously infected had to be discarded. The $F_1$ plants were grown in Maine. Due to scab and unfavorable climatic conditions a large number of seeds failed to grow and the number of $F_1$ plants is not as large as desired in many cases.

In making crosses only six or eight spikelets were left on a head and the central flowers of each spikelet were removed. The remaining flowers were emasculated and the head wrapped in tissue paper both before and after pollinating. There was thus no chance for wind-pollination to occur.

In studying the behavior of the endosperm, the weight of individual grains of the parents and of fertile and sterile $F_1$ hybrids was determined. A glass scale used for this purpose permitted accurate and rapid work.

The degree of sterility in the hybrids was determined in two ways. (1) The number of grains per spikelet was obtained for parents and $F_1$ plants. (2) The percentage of sterile pollen was determined. Pollen counts were obtained by taking anthers from several plants and shaking out the pollen on a slide smeared with albumin fixative. A drop of lactic acid was added, the preparation covered and sealed with balsam. Preparations made in this way were in perfect condition after six months. In addition to differences in contents of pollen grains there was considerable variation in the size of pollen grains. In order to obtain accurate data pollen grains of parents and $F_1$ hybrids were measured with a Zeiss ocular micrometer.

## THE $F_1$ ENDOSPERM IN FERTILE AND STERILE HYBRIDS

As in maize the endosperm resulting from the immediate cross contains chromosomes contributed by both parents due to double fertilization (SAX 1918). In wheat the embryo sac is derived from a single megaspore (JENSEN 1918). This megaspore is one of four megaspores resulting from the reduction divisions and contains the haploid number of chromosomes. Since in wheat the embryo sac is derived from a megaspore, the egg nucleus and the two polar nuclei each contain haploid chromosomes of identical constitution. The $F_1$ endosperm resulting from the immediate cross has the same chromosome constitution as the $F_1$ plant, plus an extra set of maternal chromosomes. The grain resulting from the immediate cross will be called the $F_1$ grain and the endosperm the $F_1$ endosperm. This grain contains the embryo of the $F_1$ plant. The grains borne on the $F_1$ plant will be designated $F_2$ grains and the endosperm the $F_2$ endosperm.

When the hybrid grains ($F_1$ endosperm) were examined, it was found that all combinations which later proved to be partially sterile had small wrinkled grains, while the fertile combinations had plump grains often larger than those of the female parent. Since the development of size and shape of grain is normally dependent on the female parent plant (ENGLEDOW 1920), $F_1$ grains were compared only with grains of the female parent.

For purposes of comparison, grains of parent varieties were selected at random from the cereal garden. In order to make the comparison with the hybrid grains more exact, a number of heads were emasculated and pollinated with pollen from another plant of the same variety. The number of grains set was too small in most cases, for accurate conclusions, so a field selection was made instead. The grains of parents of fertile crosses and sterile crosses were weighed and the means and standard deviations determined. The grains were weighed to the nearest 5-milligram unit but are recorded in classes of 5 mg. Table 1 gives the data obtained.

The Emmer group has somewhat larger grains than the *vulgare* group. The grain weight of Hybrid 143 was unusually low due to lateness in maturing and to rust infection. In comparing $F_1$ hybrid grain with parents only the female parent was used for reasons already stated. The average weight of the $F_1$ grains which produce partially sterile $F_1$ plants is less than the average grain weight of the female parent. In all cases these grains were wrinkled. The $F_1$ grains which later produced fertile $F_1$ plants are as a rule larger than grains of the female parent. The weights of $F_1$ grains are no more variable than the weight of grain of the parents.

TABLE 1

*Weight of grains, of parents and $F_1$ endosperm.*

| | WEIGHT IN MILLIGRAMS (5-MG UNITS) | | | | | | | | | | | | | NUMBER | MEAN | $\sigma$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2.5–7.5 | 7.5–12.5 | 12.5–17.5 | 17.5–22.5 | 22.5–27.5 | 27.5–32.5 | 32.5–37.5 | 37.5–42.5 | 42.5–47.5 | 47.5–52.5 | 52.5–57.5 | 57.5–62.5 | 62.5–67.5 | 67.5–72.5 | | | |
| Parents: | | | | | | | | | | | | | | | | |
| Kubanka | | | | 1 | 3 | 8 | 8 | 8 | 6 | 8 | 2 | 2 | | | 46 | 39.9±1.0 | 9.7±0.7 |
| Alaska | | | 5 | 13 | 21 | 12 | 4 | 2 | | | | | | | 57 | 25.3±0.6 | 5.9±0.4 |
| Bluestem | | 2 | 2 | 5 | 9 | 16 | 11 | 8 | 1 | | | | | | 54 | 29.8±0.7 | 7.8±0.5 |
| Amby | | | 1 | 3 | 3 | 3 | 9 | 5 | 8 | 6 | 3 | | | | 41 | 38.6±1.1 | 10.4±0.8 |
| Hybrid 143 | | 20 | 26 | 14 | 11 | 1 | 1 | | | | | | | | 73 | 16.6±0.5 | 5.7±0.3 |
| Polish | | | | 2 | 8 | 8 | 11 | 13 | 7 | 6 | 2 | 3 | 2 | | 62 | 39.1±1.0 | 10.9±0.7 |
| Marquis | | | 4 | 6 | 9 | 11 | 8 | 4 | 2 | | | | | | 44 | 28.8±0.8 | 7.9±0.6 |
| Sterile crosses: | | | | | | | | | | | | | | | | |
| Bluestem × Kubanka | | | | 5 | 2 | 2 | | | | | | | | | 9 | 23.3±0.9 | 4.1±0.7 |
| Kubanka × Bluestem | | | 3 | 6 | 10 | 8 | 5 | 1 | | | | | | | 33 | 26.4±0.8 | 6.3±0.5 |
| Bluestem × Alaska | | 2 | 7 | 20 | 22 | 8 | | 1 | | 1 | | | | | 61 | 23.1±0.6 | 6.4±0.4 |
| Alaska × Bluestem | | | 3 | 6 | 2 | 1 | 2 | | | | | | | | 14 | 22.5±1.1 | 6.5±0.8 |
| Kubanka × Amby | 1 | 1 | 4 | 7 | 10 | 5 | 4 | 4 | 2 | | | | | | 38 | 26.5±1.0 | 9.4±0.7 |
| Amby × Kubanka | | 10 | 18 | 15 | 3 | 1 | | | | | | | | | 47 | 16.5±0.5 | 4.7±0.3 |
| Kubanka × Hybrid 143 | | | 2 | 10 | 23 | 29 | 13 | 1 | 2 | 3 | 2 | | | | 85 | 29.7±0.6 | 7.9±0.4 |
| Hybrid 143 × Kubanka | 2 | 17 | 17 | 14 | 5 | | 1 | | | | | | | | 56 | 15.7±0.5 | 5.8±0.4 |
| Alaska × Amby | | 1 | 1 | 3 | 8 | 5 | 1 | | | | | | | | 19 | 24.8±0.9 | 5.8±0.5 |
| Amby × Alaska | 1 | 4 | 14 | 18 | 4 | 1 | 1 | | | | | | | | 43 | 18.2±0.5 | 5.4±0.4 |
| Alaska × Hybrid 143 | | 4 | 10 | 13 | 9 | 7 | 12 | 6 | | | | | | | 61 | 25.4±0.8 | 9.0±0.6 |
| Hybrid 143 × Alaska | 3 | 44 | 32 | 23 | 6 | 2 | | | | | | | | | 110 | 14.6±0.4 | 5.3±0.3 |
| Polish × Hybrid 143 | | | 1 | 3 | 2 | 1 | | | | | | | | | 7 | 22.2±1.1 | 4.3±0.8 |
| Hybrid 143 × Polish | | 10 | 17 | 21 | 5 | | | 1 | | | | | | | 54 | 17.4±0.5 | 5.4±0.4 |
| Marquis × Kubanka | | 1 | 7 | 30 | 29 | 1 | 2 | | | | | | | | 70 | 22.0±0.3 | 3.8±0.2 |
| Marquis × Alaska | | 3 | 11 | 9 | 6 | 1 | | | | | | | | | 30 | 18.5±0.6 | 5.0±0.5 |
| Marquis × Polish | | 2 | 10 | 6 | 4 | | 2 | | | | | | | | 24 | 19.2±0.9 | 6.4±0.6 |
| Fertile crosses: | | | | | | | | | | | | | | | | |
| Bluestem × Amby | | | | | | | 1 | | 4 | 9 | 9 | 3 | | | 26 | 51.6±0.7 | 5.6±0.5 |
| Amby × Bluestem | | | 3 | 2 | 5 | 5 | 3 | 2 | 4 | 2 | | | | | 26 | 31.7±1.4 | 10.6±1.0 |
| Bluestem × Hybrid 143 | | | | | | | 1 | 2 | 1 | 7 | 15 | 5 | 2 | | 33 | 53.5±0.8 | 6.5±0.6 |
| Hybrid 143 × Bluestem | | 11 | 10 | 18 | 9 | 4 | 4 | 1 | | | | | | | 57 | 20.1±0.7 | 7.6±0.5 |
| Amby × Hybrid 143 | | | | | | | 5 | 7 | 12 | 6 | 1 | | | | 31 | 43.6±0.6 | 5.3±0.5 |
| Hybrid 143 × Amby | | | 1 | 2 | 13 | 8 | 6 | 4 | | | | | | | 34 | 29.2±0.7 | 6.1±0.5 |
| Marquis × Amby | | | 2 | 4 | 4 | 6 | 12 | 3 | 4 | 4 | | | | | 39 | 33.6±1.0 | 9.5±0.7 |
| Marquis × Hybrid 143 | | | 1 | 4 | 3 | 5 | 3 | 3 | 2 | | | | | | 21 | 30.3±1.3 | 8.5±0.9 |
| Kubanka × Alaska | | | 1 | 2 | 2 | 8 | 12 | 18 | 39 | 25 | 20 | 15 | 14 | 6 | 162 | 48.2±0.6 | 11.1±0.4 |
| Alaska × Kubanka | | | | 2 | 7 | 13 | 10 | 10 | 7 | 1 | | | | | 50 | 34.4±0.7 | 7.3±0.5 |
| Kubanka × Polish | | | | 2 | 2 | 3 | 8 | 10 | 18 | 8 | 4 | 1 | | | 56 | 42.0±0.8 | 8.5±0.6 |

Table 2 was prepared to facilitate comparisons between grains of parents and hybrids. The average weight of grain of each parent is given as well as the difference between the grain weight of the female parent and the weight of the hybrid grain ($F_1$ endosperm). For example Kubanka selfed resulted in grains weighing 39.9 mg; Kubanka pollinated with Alaska pollen produced $F_1$ grains which weigh 8.3 mg more than Kubanka grains;

TABLE 2

*Showing mean weight of grains from parents and differences between mean weight of hybrid grains ($F_1$ endosperm) and mean weight of grains from ♀ parent, E = Emmer group; V = vulgare group. Weight in milligrams.*

| ♀ \ ♂ | Alaska E | Kubanka E | Polish E | Bluestem V | Marquis V | Hybrid 143 V | Amby V |
|---|---|---|---|---|---|---|---|
| Alaska E | 25.3±0.6 | +9.1±0.9 | | −2.8±1.3 | | +0.1±1.0 | −0.5±1.1 |
| Kubanka E | +8.3±1.2 | 39.9±1.0 | +2.1±1.3 | −13.5±1.3 | | −10.2±1.2 | −13.4±1.4 |
| Polish E | | | 39.1±1.0 | | | −16.9±1.5 | |
| Bluestem V | −6.7±0.9 | −6.5±1.1 | | 29.8±0.7 | | +23.7±1.1 | +21.8±1.0 |
| Marquis V | −10.3±1.0 | −6.8±0.8 | −9.6±1.2 | | 28.8±0.8 | +1.5±1.5 | +4.8±1.3 |
| Hybrid 143 V | −2.0±0.6 | −0.9±0.7 | +0.8±0.7 | +3.5±0.9 | | 16.6±0.5 | +12.6±0.9 |
| Amby V | −20.4±1.2 | −22.1±1.2 | | −6.9±1.8 | | +5.0±1.3 | 38.6±1.1 |

Kubanka pollinated with Bluestem pollen produced $F_1$ grains which weigh 13.5 mg less than Kubanka grains. All of the $F_1$ grains in the Emmer group are heavier than the grains of the female parent. The difference is hardly significant, however, in case of Polish × Kubanka. In the *vulgare* group all crossed grains are significantly larger than those of the female parent with the exception of Amby × Bluestem. The reciprocal cross

on the other hand produced grains much larger than those of the female parent. Both of these crosses, however, behave in a very unusual manner in the first generation which will be described later. Most of the partially sterile crosses result in grains significantly smaller than those of the female parent. In no case is the average grain weight significantly larger than that of the female parent. One of the most striking cases is that of Bluestem crossed with members of the Emmer group and with members of the *vulgare* group. When selfed the average grain weight is 29.8 mg; when pollinated with pollen from the Emmer group the average weight is 23.2 mg and when pollinated with pollen from the *vulgare* group the average weight is 52.5 mg. It appears that there is some effect of hybrid vigor in the $F_1$ endosperm of fertile crosses and a considerable decrease in average grain weight in the case of partially sterile crosses.

The technique used in making crosses may have some effect on the size the grain attains. Part of the head is cut off and the central florets of the remaining 6 to 8 spikelets are removed. Such mutilation may be injurious, but at the same time there are fewer grains to be developed per head. Certainly the technique used in crossing could not account for the decrease in weight of grain ($F_1$ endosperm) in sterile crosses and at the same time account for an increase in weight of grain in fertile crosses. Crossed grains compared with parent grains from plants subject to the same technique also show increased weight of grain of fertile crosses and decreased weight in case of grains of partially sterile crosses.

Crosses made in 1920 show that Spelt (*T. spelta*) crossed with the *vulgare* group results in plump grain and when crossed with members of the Emmer group result in small shriveled grains. Emmer (*T. dicoccum*) crossed with members of the Emmer group produced plump large grains while pollinated with pollen from the *vulgare* group only small shriveled grains resulted.

## HYBRID VIGOR IN FERTILE AND PARTIALLY STERILE CROSSES

In order to determine if sterility has any effect on vegetative vigor, the parent and $F_1$ plants were grown under as nearly uniform conditions as possible. Due to scab infection and unfavorable climatic conditions a comparatively small number of plants survived and the numbers were too low for significant comparison in many cases. By grouping the parent plants, and $F_1$ plants, respectively, comparisons can be made between the two parent groups and between fertile and sterile hybrids (table 3). The grouping of varieties and crosses in this way is subject to some error, but it is believed that the conclusions to be drawn are correct.

The $F_1$ plants of both the fertile and partially sterile $F_1$ hybrids are taller than the parent plants. The fertile $F_1$ hybrids resulting from crosses of species in the Emmer group are slightly taller than the average of the parents. The fertile crosses between *T. vulgare* and *T. compactum* result in $F_1$ plants which average 7.8 ± 1.8 cm taller than the average of the parents. The parents, of course, vary considerably in height since *T. compactum* has short culms as well as club heads. It may appear that the increased height of the $F_1$ hybrids as compared with the average of the parents is due to dominance of the taller parent. The $F_1$ head shape is intermediate however, and since length of head and length of straw are positively

TABLE 3

*Height of parent groups, fertile $F_1$ hybrids, partially sterile $F_1$ hybrids, and completely sterile $F_1$ hybrids.*

| PARENTS AND $F_1$ HYBRIDS | FREQUENCIES OF HEIGHTS IN 5-CENTIMETER CLASSES | | | | | | | | | | | | | | | | | | NUMBER | MEAN | σ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 55–60 | 60–65 | 65–70 | 70–75 | 75–80 | 80–85 | 85–90 | 90–95 | 95–100 | 100–105 | 105–110 | 110–115 | 115–120 | 120–125 | 125–130 | 130–135 | 135–140 | 140–145 | | | |
| Emmer group | | | | | 1 | 3 | 5 | 16 | 12 | 19 | 17 | 8 | 1 | 1 | 1 | | | | 84 | 100.7±0.7 | 9.2±0.5 |
| *Vulgare* group | 1 | 5 | 6 | 15 | 10 | 13 | 14 | 20 | 8 | 3 | 9 | 1 | | | | | | | 105 | 85.3±0.8 | 12.7±0.6 |
| Emmer × Emmer | | | | | 1 | 3 | 4 | 4 | 9 | 7 | 9 | 5 | 3 | 6 | 1 | | | | 52 | 103.6±1.1 | 12.2±0.8 |
| *Vulgare* × *vulgare* | 3 | | 3 | 3 | 3 | 4 | 5 | 1 | 5 | 9 | 7 | 8 | | | | | | | 51 | 93.1±1.6 | 16.4±1.1 |
| *Vulgare* × Emmer* | | | | 2 | 2 | 8 | 11 | 14 | 19 | 16 | 17 | 17 | 10 | 8 | 2 | 6 | | | 132 | 103.5±0.8 | 13.7±0.6 |
| Alaska × Einkorn† | | | | | | | | | | | | | 1 | 1 | 1 | | 1 | 1 | 5 | 129.5±2.8 | 9.3±2.0 |

* Partially sterile.

† Almost completely sterile.

correlated (FRUWIRTH 1910) the height of $F_1$ plants resulting from a cross of *T. compactum* × *T. vulgare* should be intermediate unless other factors are involved. The $F_1$ plants resulting from crosses of members of the *vulgare* group × members of the Emmer group are partially sterile. The height of these plants, however, is 10.5 ± 1.1 cm taller than the average of the parent groups. Only five plants of Alaska × Einkorn were grown. These $F_1$ plants although totally sterile exceed the average height of the parents by 34.8 ± 3.0 cm. The average height of Alaska was 102.3 ± 1.1 cm while Einkorn plants were 87.0 ± 1.0 cm tall. It is evident that the partially or completely sterile $F_1$ plants often exceed the parents in vegetative vigor even though they originate from small wrinkled grains.

LETHAL FACTORS IN WHEAT CROSSES

The cross of Bluestem × Amby and the reciprocal might be expected to behave the same as other crosses in the *vulgare* group. These varieties when crossed with other members of the *vulgare* group produced plump grain ($F_1$ endosperm) and the resulting $F_1$ plants are wholly fertile. Twenty six grains of the cross Bluestem × Amby and 26 grains of the reciprocal cross were planted. Twelve grains of each cross were planted in the greenhouse in Illinois. They all germinated and appeared to develop normally until they reached the height of 15 to 20 cm. They then ceased to increase in height although they were vigorous and stooled excessively, often as many as 40 or 50 primary culms were formed, but in no case did a true culm develop. The plants were observed for 5 months but did not develop further. The behavior of this cross was the same when planted in the field although the plants died before the growing season was ended. The unusual growth of this cross might be attributed to factors for winter habit, but the upright habit of growth and excessive stooling are not characteristic of the early stages of development of winter wheat. In all other cases both the parent and $F_1$ plants developed normally and the $F_1$ hybrids even exceeded the parents in vegetative vigor. The uniform behavior of the $F_1$ plants of Bluestem × Amby and reciprocal, both in the greenhouse and under field conditions precludes any argument that external factors were involved. It is evident that in this cross we have factors which prevent the hybrid plant from developing beyond a very definite stage. This case can be analyzed by crossing the $F_1$ of Amby × Marquis with Bluestem.

STERILITY IN THE $F_1$ HYBRIDS

The degree of sterility of various crosses was determined by pollen counts and by the grain set per spikelet. The pollen grains were mounted in lactic acid and examined under a microscope. All grains which appeared to be empty were classed as poor pollen. A cytological examination would undoubtedly reveal other abnormal conditions and would increase the counts of poor pollen grains. In shaking out the mature pollen grains the heavier pollen grains will come out more readily than the small shriveled ones so that the absolute degree of sterility cannot be determined by this method. For comparative purposes the method used is considered satisfactory. The following table shows the amount of pollen sterility and grain set for parents and $F_1$ hybrids.

With two exceptions the grains set per spikelet on the parent plants range from 1.85 to 2.91. The grain set in case of Hybrid 143 was unusually low due to late maturity and unfavorable climatic conditions for the variety. The Einkorn used normally sets only one grain per spikelet so that 0.97 grains per spikelet is nearly a perfect set.

TABLE 4

*Sterility in parent and $F_1$ plants as indicated by grain set and pollen development.*

| VARIETY OR CROSS ($F_1$) | GRAIN SET | | | POLLEN | | |
|---|---|---|---|---|---|---|
| | Spikelets | Grains | Grains per spikelet | Total count | Poor pollen | % poor pollen |
| Polish | 345 | 638 | 1.85 | 1016 | 16 | 1.6 |
| Kubanka | 398 | 1159 | 2.91 | 1019 | 19 | 1.9 |
| Alaska | 247 | 478 | 1.93 | 1015 | 15 | 1.5 |
| Einkorn | 551 | 535 | 0.97 | 1003 | 3 | 0.3 |
| Bluestem | 402 | 1036 | 2.58 | 1009 | 9 | 0.9 |
| Amby | 284 | 717 | 2.52 | | | |
| Hybrid 143 | 179 | 269 | 1.49 | 770 | 10 | 1.3 |
| Marquis | 380 | 1039 | 2.73 | 1011 | 11 | 1.1 |
| Semi-sterile crosses: | | | | | | |
| Bluestem × Alaska | 935 | 1139 | 1.22 | | | |
| Amby × Kubanka | 88 | 45 | 0.51 | | | |
| Kubanka × Hybrid 143 | 59 | 29 | 0.49 | 759 | 94 | 12.4 |
| Hybrid 143 × Kubanka | 301 | 136 | 0.45 | 838 | 135 | 16.1 |
| Hybrid 143 × Alaska | 591 | 616 | 1.04 | 676 | 103 | 15.2 |
| Hybrid 143 × Polish | 328 | 190 | 0.58 | 1144 | 176 | 15.4 |
| Marquis × Kubanka | 182 | 181 | 0.99 | 737 | 125 | 17.0 |
| Marquis × Alaska | 110 | 211 | 1.92 | | | |
| Marquis × Polish | 96 | 161 | 1.68 | 834 | 130 | 15.6 |
| Kubanka × Bluestem | 127 | 58 | 0.46 | 1919 | 413 | 21.5 |
| Kubanka × Marquis | 46 | 65 | 1.41 | 469 | 54 | 11.5 |
| Totally sterile: | | | | | | |
| Alaska × Einkorn | 127 | 0 | 0 | 622 | 608 | 97.7 |
| Fertile crosses: | | | | | | |
| Bluestem × Hybrid 143 | 425 | 1033 | 2.43 | 1004 | 4 | 0.4 |
| Hybrid 143 × Bluestem | 290 | 827 | 2.85 | | | |
| Hybrid 143 × Amby | 90 | 304 | 3.37 | | | |
| Marquis × Amby | 47 | 129 | 2.74 | | | |
| Marquis × Hybrid 143 | 54 | 175 | 3.24 | | | |
| Kubanka × Alaska | 347 | 1239 | 3.57 | 708 | 9 | 0.9 |
| Alaska × Kubanka | 127 | 463 | 3.64 | | | |
| Kubanka × Polish | 381 | 994 | 2.61 | 1012 | 12 | 1.2 |

The proportion of poor pollen grains as indicated by small empty pollen grains is only about 1 percent in the parent plants. Lack of vigor, as in

case of Hybrid 143, seems to have no effect on the normal development of pollen grains. Pollen counts give more uniform results in regard to fertility than do the grains per spikelet.

All crosses between members of the two general groups,—Emmer group and *vulgare* group,—resulted in partially sterile $F_1$ plants. As indicated in table 4 the grains set range from 0.45 to 1.92 per spikelet. In all cases the grains borne on partially sterile $F_1$ plants varied greatly in size. Many grains were small and wrinkled like the $F_1$ endosperm, while others were even larger than the parent grains. The amount of sterility as indicated by percentage of poor pollen grains does not vary greatly in different crosses. The number of poor pollen grains in partially sterile crosses is about 15 percent as compared with approximately 1 percent for the parents. Attention is called to the fact that in the crosses Kubanka × Hybrid 143 and reciprocal and Kubanka × Marquis and reciprocal the number of grains per spikelet is greater, and the pollen sterility is less, when a member of the Emmer group is used as the female parent. The same relation is found in other crosses to be reported later. The cross of Marquis × Alaska is nearly as fertile as the parents. Unfortunately pollen counts were not made. It seems rather paradoxical to say that a partially sterile hybrid is as fertile as one of the parents. There is no question that the cross is partially sterile and would produce all degrees of sterility in the second generation. The grains on the $F_1$ plant of this cross vary from small shriveled grains to large plump grains. This extreme variation in size of grains on the $F_1$ plant is characteristic only in partially sterile crosses.

The $F_1$ plants of Alaska × Einkorn produce no grain and nearly all of the pollen grains are obviously poor. It is probable that none of the pollen grains are functional and that the few grains set in occasional instances are due to wind-pollination. It is assumed therefore that the female gametes are more fertile than the male gametes as is the case in most partially sterile hybrids of plants and animals.

The crosses of varieties within each general group of species give fertile $F_1$ plants. The grains set per spikelet are even greater than in the parent plants due to the vigor of the $F_1$ plants. The percentage of poor pollen is no greater than in the parents.

### SIZE OF POLLEN GRAINS IN PARENTS AND HYBRIDS

While examining the pollen of parents and $F_1$ hybrids it was found that considerable difference existed in the size and uniformity of the pollen grains. Measurements were made with a Zeiss micrometer. Random

TABLE 5

*Diameter of pollen grains of parent and $F_1$ plants. "Good" or "poor" after cross indicates that pollen is apparently good or poor.*

| VARIETY OR CROSS | DIAMETER IN MICRONS | | | | | | | | | | | | | | | | | | | | | NUMBER | MEAN | σ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 33.5–35.5 | 35.5–37.5 | 37.5–39.5 | 39.5–41.5 | 41.5–43.5 | 43.5–45.5 | 45.5–47.5 | 47.5–49.5 | 49.5–51.5 | 51.5–53.5 | 53.5–55.5 | 55.5–57.5 | 57.5–59.5 | 59.5–61.5 | 61.5–63.5 | 63.5–65.5 | 65.5–67.5 | 67.5–69.5 | 69.5–71.5 | 71.5–73.5 | 73.5–75.5 | | | |
| Kubanka | | | | | 1 | 2 | 2 | 2 | 6 | 10 | 18 | 17 | 20 | 8 | 2 | 1 | | 1 | | | | 90 | 55.6±0.3 | 4.3±0.2 |
| Marquis × Kubanka, good | | | | | | | | 1 | 3 | 4 | 9 | 6 | 6 | 14 | 12 | 13 | 12 | 5 | 9 | 5 | 7 | 106 | 63.1±0.4 | 6.5±0.3 |
| Marquis × Kubanka, poor | | | | 1 | 1 | | 5 | 2 | 3 | 4 | 3 | 4 | | | | | | | | | | 23 | 48.6±0.6 | 4.5±0.4 |
| Kubanka × Marquis—good | | | | | | 4 | 9 | 6 | 7 | 9 | 8 | 5 | 8 | 7 | 11 | 5 | 1 | 3 | 1 | | | 84 | 53.7±0.5 | 6.7±0.3 |
| Marquis | | | | | | | 1 | 1 | | | 1 | 8 | 6 | 21 | 16 | 18 | 12 | 7 | 1 | | | 92 | 60.3±0.3 | 4.1±0.2 |
| Alaska | | | | | | | 1 | 2 | 5 | 2 | 8 | 15 | 13 | 21 | 13 | 6 | 5 | 1 | 2 | | | 94 | 57.1±0.3 | 4.7±0.2 |
| Alaska × Einkorn—poor | 5 | | 6 | 19 | 20 | 11 | 5 | | | | | | | | | | | | | | | 66 | 39.7±0.2 | 2.6±0.2 |
| Einkorn | | | | | 1 | 2 | 7 | 8 | 7 | 11 | 17 | 16 | 8 | 2 | 2 | 1 | | | | | | 82 | 51.5±0.3 | 4.5±0.3 |
| Hybrid 143 | | | | | | | | 1 | 1 | | 1 | 4 | 12 | 21 | 16 | 10 | 15 | 5 | | | | 86 | 60.1±0.3 | 3.8±0.2 |
| Alaska × Kubanka—good | | | | | | 2 | 3 | 5 | 4 | 9 | 11 | 14 | 11 | 18 | 9 | 14 | 8 | 4 | 2 | 3 | | 117 | 57.0±0.4 | 6.3±0.3 |
| Hybrid 143 × Alaska—good | | | | | | 1 | | 1 | 4 | 8 | 9 | 8 | 10 | 19 | 4 | 7 | 8 | 2 | 1 | | | 82 | 56.9±0.4 | 5.2±0.3 |
| Kubanka × Bluestem—good | | | | | | 2 | 2 | 2 | 2 | 12 | 8 | 5 | 5 | 7 | 10 | 3 | 8 | 7 | 4 | | | 77 | 57.3±0.5 | 7.1±0.4 |

samples were taken by measuring the pollen grains in each of a series of fields taken in definite areas across the slide. Table 5 shows the size and standard deviation of pollen grains of several of the parents and hybrids.

The pollen grains of Marquis and Hybrid 143 (*vulgare* group) have an average diameter of 60.2 microns. They are significantly larger than the pollen grains of Alaska and Kubanka (Emmer group) which have an average diameter of 56.4 microns. Einkorn, which belongs to a third sterility group, has pollen grains much smaller than either of the other two groups. The average diameter of Einkorn pollen grains is only 51.5 microns. The mean volume of the pollen grains for the three groups expressed in thousands of cubic microns assuming that the pollen grains are perfectly spherical, is 114 for the *vulgare* group, 94 for the Emmer group and 72 for the Einkorn. The obviously poor pollen grains in sterile or partially sterile crosses are much smaller than the good pollen grains of the parents or the hybrids. The good pollen grains of both fertile and partially sterile hybrids are about the same size as parent pollen grains with one exception. The pollen grains of the $F_1$ of Marquis $\times$ Kubanka are even larger than pollen grains of Marquis.

The variability in size of pollen grains is greater in the $F_1$ hybrids than in the parents. In all cases with the possible exception of Hybrid 143 $\times$ Alaska the difference between the standard deviation of the parents' pollen grains and those of the $F_1$ hybrids are significant. In the case of Marquis $\times$ Kubanka, there is a difference of $2.3 \pm 0.4$ and in the reciprocal cross the difference between the standard deviation of the pollen grains of the $F_1$ plant and the standard deviation of the average size of pollen grains of the parents is $2.5 \pm 0.4$. In the case of partially sterile hybrids only the apparently good pollen grains were measured except in the cases mentioned. If these poor pollen grains were included the standard deviation of the size of pollen grains of the partially sterile $F_1$ plants would be considerably increased, as the obviously poor pollen grains are usually small. In the fertile cross Alaska $\times$ Kubanka the difference in standard deviation between parent and $F_1$ pollen grains is $1.8 \pm 0.4$. The probability that this difference is statistically significant is over 400 to 1.

## CONCLUSIONS

Crosses of wheat species which produce sterile or partially sterile $F_1$ hybrids also produce small wrinkled $F_1$ grains. Fertile crosses produce large plump grains often larger than the grains of the female parent. Both fertile and sterile or partially sterile $F_1$ plants are usually more vigorous than the parents. Sterile combinations which produce small wrinkled

$F_1$ endosperms produce large vigorous $F_1$ plants. The chromosome constitution of the $F_1$ endosperm and the $F_1$ plant is identical with the exception that the $F_1$ endosperm has an extra set of maternal chromosomes. The individual chromosomes are identical and carry the same factors.

It is evident that groups of chromosomes which are incompatible in gamete formation can function normally in the vegetative development of the plant so long as the paternal and maternal sets of chromosomes are intact. In partially sterile crosses the addition of an extra set of maternal chromosomes in case of the endosperm is correlated with abnormal development. In fertile combinations the extra set of maternal chromosomes in the endosperm causes no abnormal growth, unless endosperm formation itself is considered abnormal. In fertile wheat crosses we get hybrid vigor in both $F_1$ endosperm and $F_1$ plant as in corn. In partially sterile crosses, however, we get a reduced development of the $F_1$ endosperm and yet the $F_1$ plant is unusually vigorous. Apparently the hybrid endosperm is not always adapted to the needs of the hybrid embryo as JONES (1918) has suggested. In sterile or partially sterile wheat hybrids where the chromosomes are incompatible in gamete formation the behavior of the 3x chromosomes in the $F_1$ endosperm development is not comparable with the behavior of the 2x chromosomes in the corresponding $F_1$ plant development.

The degree of sterility of a wheat hybrid may be determined by the amount of grain set on the $F_1$ plant and by the amount of aborted pollen. In addition a sterile or fertile cross can be recognized at once by the size and appearance of the $F_1$ grain resulting from the immediate cross. Sterile or partially sterile crosses produce small wrinkled $F_1$ grains, while fertile crosses produce grains as large or larger than those of the maternal parent. Comparisons are made only with the grains of the female parent because the $F_1$ grains are also borne on the female parent and size and shape of grain is normally dependent on the mother plant only.

The sterility relationships of wheat species are in accord with SCHULZ'S taxonomic classification (see VON TSCHERMAK 1914) and with VAVILOV'S (1914) classification in respect to disease resistance. The results of the present investigation as well as other work on sterility are not in complete agreement with VON TSCHERMAK'S (1914) classification. VON TSCHERMAK classes *T. dicoccum* × *T. durum*, *T. turgidum* and *T. polonicum* as weakly fertile. *T. durum* and *T. turgidum* × *T. vulgare* and *T. compactum* are classed as fertile crosses. The first group has been found to be completely fertile and the second group in all cases has proved to give partially sterile $F_1$ hybrids.

In making general statements regarding the behavior of wheat species it must be remembered that species limits are arbitrary and that in many cases there is a more or less continuous series between one species and another. This overlapping of species is especially true in the species *T. durum* and *T. turgidum* and also in *T. vulgare* and *T. compactum*. *T. compactum* might well be classed with *T. vulgare* both in respect to taxonomic similarity and genetic relationship. There is some indication that different varieties of a species may not show the same sterility relations when crossed with members of another specis. It has been shown that within a sterility group one species may not react the same as another species. For instance in the Emmer group *T. dicoccum* is more fertile with *T. vulgare* than is *T. durum* (HAYES, PARKER and KURZWEIL 1920). The three sterility groups are more distinct and clearly defined than are the species within each sterility group. The members of the Emmer group are usually bearded. They are as a rule taller, have fewer culms per plant, often have solid or pithy straw, usually have more sharply keeled glumes, and in general are more disease resistant than members of the *vulgare* group. Although there may be considerable variation in the species and varieties of each sterility group the three general groups are comparatively distinct. The general sterility classification can be made as follows:

1. Einkorn group: *T. monococcum*. All varieties cross-fertile; sterile or only slightly fertile with groups 2 and 3.
2. Emmer group: *T. dicoccum*, *T. durum*, *T. turgidum* and *T. polonicum*. All species and varieties cross-fertile; partially sterile with group 3; sterile or slightly fertile with group 1.
3. *Vulgare* group: *T. spelta*, *T. vulgare*, and *T. compactum*. All species and varieties cross-fertile; sterile with group 1; partially sterile with group 2.

The members of each group are fertile with each other and sterile or partially sterile with members of the other two groups. *T. monococcum* is completely sterile or nearly so when crossed with members of the other two groups. Crosses between members of the Emmer group with members of the *vulgare* group result in $F_1$ plants which as a rule are less than half as fertile as the parents. The sterility relationships of the above species groups are based on gametic sterility and do not apply to sterility as reported in the cross of Bluestem × Amby.

The available data indicates that the size of pollen grains varies significantly in different species of Triticum. The average volume of the pollen grains expressed in thousands of cubic microns was found to be 72 for

Einkorn, 94 for Kubanka and Alaska (Emmer group) and 114 for Marquis and Hybrid 143 (*vulgare* group). It is rather remarkable that pollen-grain size is so closely correlated with the sterility relationships of the three groups of wheat species. The size of pollen grains has little or no effect on the percentage of grain set in crossing. Crosses between members of the Emmer and *vulgare* groups are about as easy to effect as crosses within each group.

The pollen grains of both fertile and partially sterile $F_1$ plants are more variable in respect to size than pollen grains of the parents. In the partially sterile hybrids all degrees of compatible and incompatible combinations are probably present in the pollen grains. The pollen grains of the $F_1$ plant contain the haploid number of chromosomes consisting in nearly all cases, of chromosomes contributed by both parents. We may assume that some combinations are unable to function and the pollen grains abort at an early stage resulting in small empty pollen grains. Other combinations are able to function more or less favorably resulting in apparently good pollen grains of varying sizes. In the fertile hybrids and to some extent in the partially sterile hybrids, the increased variation of pollen-grain size may be attributed to differences in growth factors resulting from various combinations of maternal and paternal chromosomes. Here we have a case of variation in the haploid generation due presumably to different combinations of non-homologous maternal and paternal chromosomes.

## SUMMARY

1. The species of Triticum can be divided into three sterility groups as follows:

a. Einkorn group: *T. monococcum*. Varieties inter-fertile; sterile or only slightly fertile with groups b and c.
b. Emmer group: *T. dicoccum*, *T. durum*, *T. turgidum* and *T. polonicum*. Species and varieties inter-fertile; sterile or slightly fertile with group a; partially sterile with group c.
c. *Vulgare* group: *T. spelta*, *T. vulgare* and *T. compactum*. Species and varieties inter-fertile; sterile or slightly fertile with group a; partially sterile with group b.

2. Fertile species crosses produce $F_1$ endosperms as large or larger than those of the maternal parent. Sterile crosses produce small shriveled $F_1$ endosperms.

3. Fertile, sterile, and partially sterile $F_1$ plants resulting from species crosses equal or exceed the parents in vegetative vigor.

4. The average size of the pollen grains differs in different species and is correlated with the three sterility groups.

5. The pollen grains of the $F_1$ plants are more variable than those of the parents, due presumably to recombinations of maternal and paternal chromosomes in the haploid generation.

6. A varietal cross within the *vulgare* group produced $F_1$ plants which failed to develop beyond the rosette stage, presumably due to lethal or inhibiting factors.

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